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The effects of formulation and freezing rate on the microstructure and quality of ground beef patties

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The effects of formulation and freezing rate on the microstructure and quality of ground beef patties

by

Robert Paul Nusbaum

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TABLE OF CONTENTS

	Page
DEDICATION	vi
INTRODUCTION	1
LITERATURE REVIEW	3
Structure and Composition of Meat	3
Ice Formation in Muscle Tissue	5
The nature of freezing	5
The mechanism of ice crystal formation in muscle	6
Influence of freezing rate on ice crystal growth	8
Structural changes associated with freezing	9
Microscopic and chemical evaluation of freezing damage	12
Morphological changes of ice crystals during storage	16
Influence of Freezing on Meat Quality Traits	17
Water holding capacity and pH	18
Evaporation caused by freezing	20
Frozen product color	21
Weight changes during thawing	22
Weight loss during cooking	27
Organoleptic quality	28
Postmortem Properties of Meat	32
Chilling prerigor meat	33
Processing advantages of ground prerigor meat	34
Ice crystal formation in prerigor meat	35
MATERIALS AND METHODS	36
Experimental Design	36

	Page
Boneless Beef Trim Preparation	37
Frozen-flaked trim	37
Nonfrozen trim	37
Prerigor trim	38
Postrigor trim	38
Ground Beef Pattie Formulation	38
Freezing Treatments	39
6 Minute freezing rate	40
30 Minute freezing rate	40
80 Minute freezing rate	40
100 Minute freezing rate	41
Thawing Treatments	41
Cooking Treatments	42
Organoleptic Evaluation	42
Analytical Methods	44
Proximate analysis	44
pH	44
Water holding capacity	44
Reflectance value and color	45
DNA content of drip fluid	45
Protein content of drip fluid	46
Texture	46
Preparation of Pattie Samples for Light Microscopy (LM) Evaluation	47
Unfrozen samples	47
Frozen samples	48
Preparation of Pattie Samples for Scanning Electron Microscopy (SEM) Evaluation	48
Unfrozen samples	48
Frozen samples	50
Unfrozen, cooked samples	50
Frozen, cooked samples	50
Statistical Analysis	51

	Page
RESULTS AND DISCUSSION	52
Proximate Analysis and Sampling	52
Effects of Freezing Rate and Formulation on Color, pH, Water Holding Capacity and Freezing Shrink	52
Color	52
pH	56
Water holding capacity	58
Freezing shrink	60
Effects of Freezing Rate and Formulation on Thawing Characteristics	60
Weight gain	60
Drip loss	65
DNA content of drip fluid	70
Protein content of drip fluid	70
Effects of Freezing Rate and Formulation on Cooking Characteristics	74
Cooking from unfrozen and frozen states	74
Cooking after thawing at 5° or 20°C	76
Effects of Freezing Rate and Formulation on Sensory Characteristics	83
Formulation comparisons	83
Freezing rate comparisons	91
Effects of Freezing Rate and Formulation on Microstructure (LM and SEM) of Unfrozen, Frozen and Cooked Patties	97
Unfrozen formulation comparisons (LM and SEM)	98
Freezing rate comparisons (LM and SEM)	105
Frozen formulation comparisons (LM and SEM)	126
Unfrozen and cooked vs frozen and cooked comparisons (SEM)	144
SUMMARY	156
CONCLUSIONS	157
ACKNOWLEDGEMENTS	158

	Page
BIBLIOGRAPHY	160
APPENDIX A	170
APPENDIX B	180
Ultraviolet Absorption Measurements of DNA	181
Biuret Protein Analysis	182
Lipid Staining	183

DEDICATION

To my parents

Aleen Kuhlman
and
Daniel David Nusbaum

for all their love and support.

INTRODUCTION

The hamburger has emerged as a traditional and integral component of the American diet. In 1970 about 20% of all grainfed steer and heifer carcasses was used for ground beef. Today it is 45% with predictions of 60% by the early 1980s (Root, 1978).

Hamburger and ground beef processed under federal inspection in 1977 was in excess of 2.8 billion pounds (AMI, 1978). An additional 357 million pounds of meat patties containing extended product were also produced. Approximately 45% of all beef produced is eaten in restaurants, school cafeterias and other institutions, much of it in the form of the popular ground beef pattie. McDonald's, the worlds largest hamburger restaurant chain, serves nine million pounds of 100% pure ground beef patties each week (Root, 1978).

As ground beef pattie production increases to meet the growing demand of the fast-food industry, new knowledge in the field of ground beef handling systems is essential. One key area deals with freezing methods employed to maintain ground beef pattie quality. Another area includes the use of varying proportions of fresh (nonfrozen) and frozen meat for ground beef pattie formulations. Also, energy limitations and production inefficiencies have created current interest in the use of prerigor meat in ground beef pattie production.

The purpose of this study was to evaluate and compare the effects of six different pattie formulations and four freezing rates on freezing, thawing, cooking and sensory characteristics of ground beef patties. Light and scanning electron micrographs were taken of unfrozen, frozen and cooked pattie formulations in an attempt to investigate the effects of freezing and cooking on ground beef pattie structure. Structural differences due to freezing and/or pattie formulation were related to tested quality differences.

LITERATURE REVIEW

Structure and Composition of Meat

Muscle is a complex substance containing water, proteins, fat, carbohydrates and various cellular components. Regardless of the ultimate destiny of muscle tissue utilized by the meat industry, the final meat product will have certain characteristics that are dependent upon the chemical and physical properties of the muscle prior to processing. These preprocessing properties of the meat, together with specific processing conditions and procedures affect final product quality.

Meat consists mainly of skeletal muscle comprised of similarly structured muscle fibers which are closely held together by interstitial connective tissue. A number of these fibers make up a muscle bundle and a number of muscle bundles in turn make up the entire muscle. To understand more clearly what takes place in the freezing of meat, the structure of a normal muscle fiber or cell should first be considered.

Since the initial electron microscopy work of Huxley (1953) revealing the organization of components in skeletal muscle, the ultrastructure of the muscle fiber has been well-documented by several investigators (Huxley, 1969; Pepe, 1971; Squire, 1973; Weber and Murray, 1973). Each fiber (10-100 μm

diameter; 2-3 cm in length) is surrounded by a typical lipoprotein membrane, the sarcolemma, which is highly selective in its permeability to solutes. The space within the sarcolemma is mostly occupied by uniformly aligned cylindrical organelles called myofibrils, each about 1-3 μm in diameter. There are approximately one thousand myofibrils in each myofiber. Each myofibril is encased in a thin vesicular structure, the sarcoplasmic reticulum, which is involved in the transmission of the nervous impulse to the contractile elements. The myofibrils occupy about 74% of the total fiber volume. The fluid surrounding the myofibrils, the sarcoplasm, contains subcellular organelles and the sarcoplasmic proteins. A fibril itself contains about 16% contractile protein and about 84% water in which is dissolved small solutes such as ATP, phosphocreatine and numerous soluble enzymes (Scopes, 1970; Voyle, 1974).

The amount and distribution of fluid in the muscle is important when studying the freezing of animal tissue. According to Voyle (1974) 5-12% of the total fluid volume in a well-drained, intact postmortem muscle is located in the spaces between muscle fibers while 88-95% is found within the fiber. Of the fluid within the fiber, 22-24% of the total volume is found between the myofibrils while the remainder (66-71%) is distributed within the myofibrils.

Ice Formation in Muscle Tissue

The nature of freezing

When pure water is supercooled below 0°C ice begins to form and, in forming, liberates heat at the rate of 80 calories per gram. The temperature rises and remains at 0°C until all the water has changed to ice. When salts are present in the water, as in meat tissue, no ice appears until the temperature is below 0°C and, as ice forms, the concentration of the salts in solution increases and a lower temperature (more heat removal) is required to form more ice (Callow, 1952; Meryman, 1963). Meryman (1956) described freezing as "nothing more than the removal of pure water from solution and its isolation into biologically inert foreign bodies, the ice crystals."

A more recent and detailed explanation of changes in tissue due to freezing was offered by LaSalle (1975). He states "under freezing conditions many changes can occur such as formation of ice crystals, exosmosis, increased solubility of gases, dehydration, increased concentration of electrolytes, colloids, salts, carbohydrates, lipids, proteins, lower pH, changes in heat and electric conductivity, decreased activity of some enzymes (dehydrogenase), increased activity of other enzymes (catalase, lipase), accumulation of intermediary products, reduced intermolecular spaces, increased molecular contacts, disruption of weak hydrogen

bonds, breakdown of emulsions, formation of gels, precipitates, abnormal bonds, cross linkages, polymers, folding and distortion of large molecules, loss of cell membrane integrity, cellular invasion by toxic and mutagenic salts, eutectic concentration of all solutions, solidification, immobilization of all molecules, and drastic changes of physical, chemical and biological properties."

It is apparent that the freezing process is complex and affects muscle tissue in many different ways. The immediate effects of freezing, comprising the separation and growth of ice in the tissue and the changes that occur in the tissue during storage in the frozen state, have been investigated by numerous scientists concerned with the optimum preservation of muscle tissue as food (Hiner et al., 1945; Ramsbottom, 1947; Dunker and Hankins, 1953; Deatherage and Hamm, 1960; Ritchey and Hostetler, 1964; Smith et al., 1968; Lind et al., 1971; Berry et al., 1971; Sebranek et al., 1978).

The mechanism of ice crystal formation in muscle

Meryman (1963) and Karow and Webb (1965) published excellent reviews of the theories of ice crystal growth in biological material. A collective summary of their reviews indicates that ice crystals can develop only at a temperature that will permit the utilization of water or substances within the water to act as a center or nucleus for ice formation. The size of the nucleus that will support crystal

growth is temperature dependent; the higher the temperature, the larger the critical size. At some supercooled temperature, an existing nucleus becomes of critical size and water begins to crystallize.

When a water molecule begins to crystallize, energy is released as heat, called the latent heat of fusion. If this heat is not removed rapidly from the growing crystal, tissue temperature will remain at or near the freezing point where no further nuclei will be of critical size. Therefore, in slow freezing, only a few extracellular crystals will grow to very large size consuming all the water available for freezing in the tissue.

If the removal of heat is rapid, as in fast freezing, the temperature of the tissue will continue to fall and more nuclei will become of critical size, producing more and smaller ice crystals. With increasing rates of cooling, the tendency for extracellular crystal formation diminishes and ice crystals begin to appear inside the cells. The final result is a large number of small crystals of assorted sizes. Karow and Webb (1965) reported that the critical radius at -15°C is 25Å and at -100°C is "infinitesimal."

Thus, it appears that not only freezing temperature, but the rate of freezing within the tissue greatly affects size and distribution of ice crystals in the tissue.

Influence of freezing rate on ice crystal growth

A general theory widely accepted today states that ice crystal formation occurs inside the cell as a result of "rapid" freezing, but the crystals form outside the cell in "slow" freezing (Love, 1966). Richardson and Scherubel (1908) described for the first time the histological appearance of slowly frozen beef muscle, showing that the water separated out from the muscle fibers as crystals of ice leaving the fibers dehydrated and shrunken. At that time they were unaware of intracellular ice crystal formation. Early German work cited by Dubois and Tressler (1940) and Love (1966) found that fish fillets frozen rapidly contained many intracellular needles of ice lying parallel with the long axis of the cell. They felt that the size and distribution of ice crystals depended on the velocity of freezing.

Chambers and Hale (1932) found that ice would form on the outside of single frog muscle cells at about -1.2°C, but did not form inside until a temperature of about -1.6°C had been reached. They postulated that slow freezing yielded extracellular ice because the freezing point of extracellular fluid was higher than that of the cell contents.

"Rapid" and "slow" freezing rates were considered nebulous terms by Moran (1932) who attempted to more accurately define freezing rates. He reported experiments in which he determined the time taken for tissue to cool from

+5°C to -5°C, at which temperature 82% of the water in muscle is frozen. Moran (1932) considered "rapid" cooling to be when the tissue could pass through this 10° range without causing fiber clumping. He found the maximum time over which this cooling could occur without causing clumping when the coolant was -20°C was 48 minutes. Moran labeled this time interval the "critical time" and the rate of cooling was the "critical rate." Clumping occurred when the time taken to cool through the +5/-5° range exceeded the critical time and large extracellular ice crystals were formed. This he defined as "slow" freezing. Desrosier (1970) defined rapid freezing as the process where the temperature of the tissue passes through the zone of maximum ice crystal formation (0 to -3.9°C) in 30 minutes or less.

With a numerical definition of freezing rate, for example the degree change per minute, researchers are more able to accurately evaluate and compare their respective data (Love, 1966). Much of the literature, however, still uses only freezing temperature rather than utilizing a defined freezing rate which contributes to the confusion in this field of study.

Structural changes associated with freezing

Structural changes in meat associated with freezing rates intrigued early investigators and has continued to be

a popular area of research. When ice is formed it occupies a volume nearly 10% greater than the water from which it came and resistance to this increase in volume produces very great pressures (Callow, 1952). Early researchers were unsure if extracellular ice formation was a direct result of sarcolemmal rupture due to these pressures or if the sarcolemma was readily permeable to water during the freezing process. It appears now that either or both of these processes can occur and are directly related to speed of freezing and the type of ice crystals formed.

Many researchers reported that extracellular ice crystal growth (occurring during slow freezing) was accompanied by rupture of the sarcolemma by the cell fluids which escaped and froze. Greater damage to the cell wall after slow freezing was observed in the muscle tissue of beef (Gensho, 1950), fish (MacKay and Weld, 1926) and poultry (Dubois et al., 1942).

Other researchers have stated that the cell walls were infrequently damaged at any speed of freezing. Chambers and Hale (1932) showed a single frog cell with ice growing down the inside with an increase in the diameter of the cell with no apparent rupture of the sarcolemma. Likewise, Koonz and Ramsbottom (1939) did not observe cell damage at any speed of freezing in chicken muscle. With a slow freezing temperature (-26.1°C or above), water was lost by the fibers. When

frozen rapidly (-75.6°C) the water was frozen within the fibers. They found that a particular temperature somewhat lower than the one producing extracellular freezing induced the formation of a single, large column of ice centrally located in the fiber. Temperatures below those characterizing the single-column formation caused the formation of many intracellular ice columns. Koonz and Ramsbottom (1939) felt that the sarcolemma was readily permeable to water and not ruptured when the freezing process was prolonged as during slow freezing and that the pressure exerted upon the fibers during the growth of external ice crystals promoted the loss of intracellular water. Woolrich (1948) and later, Meryman (1956), considered that most if not all cell walls were undamaged in slow freezing.

To confuse the issue even more, Hiner et al. (1945) and Hiner and Hankins (1947) indicated that the amount of cellular damage was greater with faster rates of freezing. As rate and degree of freezing increased, with lower temperatures, less time was available for the transfer of water out of the cell. Beginning at -23.3°C, the proportion of water frozen within the cell was so great the fiber was ruptured. It was more and more extensive as the freezing temperature was lowered to -40°C and then to -80°C. At -81°C intracellular ice crystals were very extensive and small and the fibers were split longitudinally into several sections.

Microscopic and chemical evaluation of freezing damage

In an attempt to clarify much of the confusion concerning freezing damage, Rapatz and Luyet (1959) studied ice crystal patterns and the mode of ice growth in single muscle cells. They first characterized three types of ice crystal patterns that are found in muscle tissue: (1) spears of ice, (2) veils, which uniformly invade the entire cell (evanescent spherulites) and (3) clouds formed upon warming in the areas occupied by the veils (recrystallization clouds). When studying rate of ice propagation in a single fiber, they found that ice spears grow in the longitudinal direction at about 300 μm per second at -2.5°C. Transverse growth was so much less (40 μm/sec) that development of the ice phase appeared to be almost exclusively parallel to the axis of the fiber. They reported that ice crystals dimensions varied from 2 to 20 μm when frozen at -4°C and 1 to 2 μm in fibers frozen at -70°C. They also found that slow frozen fibers (-2.5°C) contained large ice masses which completely marred striations. At -10° and -15°C the particles of ice were much smaller but still of sufficient size to obliterate striations. Fibers frozen at temperatures of -45°C and below showed well-preserved striations.

A follow-up study by Menz and Luyet (1961), utilizing the transmission electron microscope, related the size, shape and location of ice particles to the intracellular

structure of the muscle fiber, particularly the myofilaments and striations. Fibers frozen at very low temperatures (-150° and -190°C) in an isopentane bath had easily recognizable A and I bands. The ice particles at these temperatures were confined within the sarcomeres. Although the myofilaments had been pushed aside by the ice particles and had aggregated into bundles separating the ice cavities, they were still held together at the Z line which had not been broken. After freezing at a higher temperature (-60°C) ice particles broke through the boundaries of the sarcomeres and disrupted the structure so that no bands or fibrils were distinguishable. They also found that when freezing at either temperature (-60° or -150°C) the ice cavities formed were smaller (200Å) near the periphery of the fiber, where the cooling rate was highest, and gradually became larger (2300Å) toward the center.

The excellent work by Rapatz and Luyet (1959) and Menz and Luyet (1961) seemed to confirm the more desirable effects of faster freezing rates. However, recent work by Koreshkov et al. (1975) reported mitochondrial fragmentation was more apparent in thawed tissue samples previously frozen with liquid nitrogen when compared to thawed samples previously frozen by slower blast freezing. These results agree with a theory put forth by Karow and Webb (1965) that rapid freezing physically deranges cell membranes promoting their

denaturation while slower freezing rates actually preserve cellular membranes. They felt that during slow freezing, water lattices are allowed to grow in the membrane forming a shield around protein structures. With rapid freezing, intracellular ice consumed not only the free water, but also portions of the bound water, weakening the lattice structure ultimately causing greater membrane disruption.

Although the theory postulated by Karow and Webb (1965) has not been proven microscopically, they were able to find significant differences in cell culture survival rates at different freezing temperatures. Thus, while faster freezing rates may harmfully affect cellular membranes when considering live cell survival, they appear to be more beneficial than slower rates for preserving total cellular integrity.

Love (1966) devised a technique to measure the effects of freezing on cell walls. He first assumed that the fluid obtained by applying pressure to muscle tissue was extracellular in origin. The basis of his technique was to analyze this fluid for deoxyribonucleic acid (DNA) which would be present only if the cell walls had been broken or ruptured by freezing. Love plotted DNA concentration against the average time to cool from 0 to -5°C. High DNA levels occurred at three rates of freezing. He labeled these DNA peaks as cell damage types A, B and C. When the freezing time (0 to -5°C) was 20-30 minutes, photomicrographs showed extensive rupture of the cell walls with intracellular freezing (Type A cell

damage). With this type of freezing, ice crystals lie near the surface of the cell, resulting in regions of weakness where rupture occurs during the expansion caused by freezing of water.

Type B damage was found to occur at approximately 100 minutes. Histologically, this was found to mark the point of differentiation between intracellular and extracellular freezing with cell walls being undamaged at slightly faster or slightly slower rates of freezing. Love (1966) attributed cause of Type B rupture to be endosmosis, a concentration of intracellular salt in one end of the cell that bursts the cell wall as pressure from forming ice pushes against it.

Type C damage was caused by the slowest freezing time (200-500 minutes). This DNA peak was accompanied by the appearance of particles of mitochondria in the extracellular fluid, indicating a severe breakdown of the cells. Love (1966) postulated that this damage was caused by the prolonged exposure of the cell to a concentrated salt solution exerting a solvent action upon the cells. Love (1966) also found that in tissue cooled from 0 to -5°C in less than 15 minutes, small ice crystals were distributed evenly throughout the cell and expansion due to freezing does not rupture the cell. When the freezing rate is slower (25 min.) Type A cell damage begins to occur.

Morphological changes of ice crystals during storage

It has been shown by several workers that the ice crystal patterns formed during freezing are not necessarily maintained in the tissue during prolonged frozen storage. Moran (1930) stated that the percentage of water in the muscle present as ice at -10°C is about 94% and gradually increases to nearly 100% at a temperature just below -20°C, which he considered the eutectic temperature of muscle. He further stated that storage temperatures above the eutectic point would allow adjoining ice crystals to merge into single, larger crystals.

Moran and Hale (1932) stored similarly frozen beef muscle for 57 and 180 days at -3.1°C and -20.3°C respectively. In each case there was a marked increase in the size of the ice crystals at the higher storage temperature. Ramsbottom and Koonz (1941), however, reported no visible change in the sizes of ice crystals in beef muscle stored for one year at -12°C. Love (1966) observed that intracellular crystals in fish muscle (5-10 μm in diameter) grew to more than double their size at -14°C, but this required 180 weeks. Menz and Luyet (1961) showed that very minute crystals (.1 - .2 μm in diameter) formed inside single cells that had been frozen rapidly at -150°C grew to many times their original size after a one minute immersion in a -15°C bath.

Recrystallization, the enlargement of ice crystals during frozen storage, appears much more likely under fluctuating temperature conditions (Love, 1962). It also is highly temperature dependent and occurs at a decreasing rate as the temperature is lowered below the freezing point (Meryman, 1956). Fennema and Powrie (1964) suggested that low and uniform temperatures would minimize recrystallization during storage of frozen foods.

Influence of Freezing on Meat Quality Traits

As previously discussed, it has been well-established that physical and chemical damage occurs during the freezing process. According to Fennema and Powrie (1964) the following factors appear to be involved: (1) damage associated with the nature and location of ice crystals, (2) mechanical damage resulting from volume changes, and (3) chemical damage caused by concentration of nonaqueous constituents. Since the rate of freezing can influence the extent of damage caused by these factors, it is appropriate to examine the relative consequences of various freezing rates as they ultimately affect meat product quality. Unfortunately, much of what is known concerning the effects of freezing applies to intact retail cuts of beef, pork, lamb and poultry with relatively little work done regarding ground meat products.

Water holding capacity and pH

An important feature of meat quality is its water holding capacity (WHC). Hamm (1960) defined WHC as "the ability of meat to hold fast its own or added water during application of any force or treatment" (processing, heating, grinding). Lack of WHC results in product weight loss and ultimately lower finished product yield (Wierbicki et al., (1957a) which is a major concern to any meat processor.

The moisture content in muscle tissue is composed of bound and free water. Hamm (1959) reported that the positive and negative charges of muscle protein directly affected the WHC of meat. He also reported that not more than 4 to 5 percent of the total water in muscle can be tightly bound to the muscle protein. Hamm (1959) concluded that the major influence of protein charges and protein structure is on the remaining or "free" water in meat, thus, WHC is more associated with the "free" water than the tightly bound water. Ockerman (1974) reported that the quantity of "free" water is related to juiciness, cooler shrink, yield on processing and drip loss.

In a study of factors affecting the water retention of beef, Swift and Berman (1959) found a direct and highly significant correlation between water retention and pH, zinc, fat content and moisture to protein ratio. McClain and

Mullins (1969) reported significant negative correlations between water loss and pH on data from three bovine muscles.

The relationship between WHC and pH shows a minimum hydration around pH 5.0 which corresponds approximately to the isoelectric point of actomyosin. A normal pH of beef is 5.4 to 5.8. Therefore, even small decreases in pH can drastically reduce WHC (Hamm, 1960).

Hamm (1959) found that grinding increases the WHC of meat by exposing more polar groups for binding with the water molecule and water is bound better when added after the meat is ground. Miller et al. (1968) reported a decrease in WHC as the fat level of the beef increased.

Freezing meat can also affect water holding capacity. Miller et al. (1968) reported that meat frozen at -34°C had higher WHC after cooking than unfrozen meat. Deatherage and Hamm (1960) reported that quick freezing (-55°C) caused a very small but significant increase in WHC of meat, probably by a mechanical loosening of tissue structure due to the formation of tiny ice crystals inside the cells. Slow freezing, at -15°C, caused a significantly small decrease of the WHC of meat, probably due to some destruction of protein structure by formation of large ice crystals between the cells. Deatherage and Hamm (1960) also concluded that quick freezing and thawing of beef resulted in no considerable denaturation of muscle protein. This was further verified by

Sang (1977) who found no difference in myofibrillar protein solubility or water holding capacity for beef patties frozen cryogenically or very slowly in still air.

Evaporation caused by freezing

As meat products are frozen, a certain amount of their moisture is lost to evaporation or dehydration commonly referred to as freezing shrink. Dunker and Hankins (1953) found that during freezing at -15°C beef samples showed greater moisture losses than did pork with fatter pork having the lowest loss. Beef frozen at -15°C had significantly greater shrink (1.5%) than beef frozen at -56°C (0.2%). They also stated that meat with the same fat content froze at the same rate in either the solid or ground form.

Aström (1971) reported that products frozen in the package lost up to 3% of their weight in evaporation, however, the moisture remained in the package as free water. He also reported that liquid nitrogen (LN_2) freezing caused shrink losses as low as 0.5% in normal freeze tunnel operation. According to Rasmussen and Olson (1972) several factors determine freezing shrink. Thicker products lost less moisture than thin products and lower product temperature immediately prior to freezing also reduced shrink loss as did lower freezing temperatures. Bucholz and Pigott (1972) froze fish fillets at -35°C and -57°C with product shrink of

1.2 and 0.8% respectively. Similarly, Cutting and Malton (1974) had greater evaporative losses of wrapped meat frozen at -8°C (1.05%) than at -30°C (0.2%). Ashby et al. (1973) froze whole hams in three freezing systems (still air, forced air and blast freezers) and found significantly different shrink losses of 1.22%, 1.09% and 0.79% respectively.

Behnke (1976) compared evaporation losses of five freezing systems, LN_2, 0.5-1%; liquid carbon dioxide (CO_2), 0.5-1%; Freon (direct contact), 0.5%; air blast, 0.6-3%; and plate freezing, 1.0-2.0%.

Tests on ground beef patties frozen by a still air freezer at -29°C showed a freezing shrink of 1.98% (Sebranek et al., 1978). In the same study, patties frozen with cryogens (LN_2 and CO_2) at -73.3°C for 8 minutes to an internal temperature of -17.8 to -20.5°C had shrinkage of less than 1%.

Frozen product color

Although food color is generally well-preserved by freezing (Fennema and Powrie, 1964; Love, 1966) the color of frozen meat products varies with the rate of freezing. Taylor (1930, 1931) reported that as the rate of freezing decreased the product color became darker. Guenther and Henrickson (1962) compared colors of steaks frozen at three rates, -9°C, -34 to -40°C and -73 to -87°C. They found that

steaks frozen at the middle rate had the most desirable color when evaluated against nonfrozen steaks. Steaks frozen at -9°C were undesirably dark while those frozen at the fastest rate were regarded as extremely pale. Similar results were reported by Jakobsson and Bengtsson (1973). Air blast freezing gave the best frozen appearance while very rapid freezing in LN_2 spray resulted in pale product color and slow freezing resulted in a darker color with the formation of ice on the product surface. Other workers have substantiated these findings with similar results (Bannister et al., 1971; Tuma, 1971; Rasmussen and Olson, 1972; Sebranek et al., 1978).

Color differences in frozen products have been attributed to ice crystal size as a result of the various freezing rates (Menz and Luyet, 1961; Judge, 1974). The numerous, small, evenly distributed ice crystals formed by faster freezing rates scatter more light than the fewer and larger crystals formed by slow freezing, thus, fast frozen meat is opaque and pale while slow frozen meat is translucent and dark (MacDougall, 1974).

Weight changes during thawing

During the freezing process, the frozen exterior transmits the heat energy released from the unfrozen core and from the freezing boundary. This transmission of heat energy occurs at a rapid rate because of the high thermal

conductivity of the forming ice. During thawing, the direction of heat flow is reversed. The initial quantities of added heat energy are absorbed rapidly due to the high thermal diffusivity of the frozen phase causing the entire tissue to rise nearly to the melting point before much surface thawing occurs. When surface thawing begins, the remainder of the additional energy needed to complete the total thawing process must be supplied through a growing layer of low-conductivity, low-diffusivity surface water. Thus, thawing takes considerably longer than freezing (Callow, 1952; Fennema and Powrie, 1964; Smith, 1970; Morley, 1974; Heldman, 1977). Fennema (1968) states that thawing, because of its prolonged nature, provides greater opportunity for damage than does freezing. Damage can result from chemical changes due to exposure to concentrated solutions, recrystallization, mechanical damage from solid/liquid transformation and microbial growth. He further states that improper thawing techniques can completely offset the advantages of superior freezing methods.

One of the disadvantages of freezing meat is the exudation of fluid (drip) on thawing. Cook et al. (1926) stated that beef which had been frozen and subsequently thawed lost a clear, reddish-colored fluid from all cut surfaces. Empey (1933) analyzed drip fluid from thawed meat and found it contained 86% water and 14% total solids con-

taining nitrogen, phosphorous, sodium chloride and ash. He also found that drip collected from thawed tissue was similar in chemical and physical composition to fluid expressed from unfrozen muscle tissue. Other components of the drip fluid as described by Sulzbacher and Gaddis (1968) included proteins, peptides, amino acids, lactic acid, purines, B-complex vitamins and various salts. The amount of drip is related to several factors according to Penny (1974). These include the condition of the meat as a result of its post-mortem treatment, pH, size of the meat product when frozen, conditions of freezing, temperature and time of storage, and conditions of thawing. When considering the collective effect of the many variables involved in drip production, two major factors emerge as ultimate determinants. One controls the extent to which the fluid, once formed, will drain from the meat. Such variables as the size and shape of the cut and ratio of cut surface to total volume are significant and seem to be the "physical" reasons contributing toward fluid exudation. The second factor, more "chemical" in nature is related to hydrogen ion concentration (pH), water holding capacity and changes in these water-protein relationships in meat brought about by freezing. A great deal of confusion exists in the literature over the proportion of total drip which can be attributed to any single factor or combinations of several factors.

As early as 1908, Richardson and Scherubel recognized the importance of thawing beef slowly as a means of reducing the amount of drip. German work in 1923 cited by Dubois and Tressler (1940) and Love (1966) confirmed these observations and suggested that drip could be reduced by freezing fast enough to prevent the separation of water from the muscle fibers. Cook et al. (1926) felt that rate of freezing appeared to be the most significant factor affecting the amount of drip. They concluded that large ice crystals formed at slow freezing rates distorted muscle fibers and damaged the sarcolemma, thus increasing drip. Moran and Hale (1932) found that time of storage at any one temperature had little effect on the amount of drip, but that increased drip occurred at the higher storage temperatures and at fluctuating storage temperatures. Work by Reay (1934) on haddock indicated that the determining factor for the amount of drip was the time spent in the range of -1 to -5°C either during freezing or thawing. Empey (1933) studied the effect of freezing and thawing rates on drip loss. Other factors investigated included age, sex and breed of animal, length of period between slaughter and freezing, and storage, composition and pH of the muscle. He showed that drip production is primarily a function of hydrogen ion concentration with the least drip being associated with muscle tissue having a pH of 6.3 or higher. Sair and Cook (1938) found that maximum drip was obtained from beef muscle having a pH of 5.2 and

as pH increased, the net drip decreased and eventually reached zero at a pH of 6.4.

Sair and Cook (1938) also found that cutting or grinding meat before freezing increased the susceptibility of the thawed meat to drip. Ramsbottom and Koonz (1939) found that irrespective of freezing temperature, there was little drip in large rib cuts where the area of cut surface was small in relation to the volume of meat. With smaller steaks, where the area of cut surface was large in relation to meat volume, the amount of drip was more dependent on freezing rate.

A later study by Ramsbottom and Koonz (1940) suggested that the time elapsing between slaughter and freezing influenced drip loss. They reported that drip decreased as time between slaughtering and freezing increased, but found only a small change in pH after the first day postmortem. Wierbicki et al. (1957a) found that drip losses of frozen aged meat were much less than those of muscle frozen in early rigor. Prior work by Wierbicki et al. (1957b) also showed that quarters of beef infused at slaughter with salt (sodium chloride) or pickling brines had little or no drip after freezing and thawing. Hamm (1960, 1977) increased water holding capacity of ground meat by adding 2-4% salt during the first hours after death.

Recent work by Footrakul (1976) suggests that thawing temperature also influences drip loss. He stated that ground

beef thawed at 4.4°C resulted in lower drip loss, firmer appearance and a higher score in juiciness and tenderness, but took a much longer time than those thawed at 21.1°C.

Weight gain of frozen meat during thawing has also been reported. Paul and Child (1937) reported weight gains in samples of beef thawed at 24°C with relative humidity at 65%. Dunker and Hankins (1953) observed weight gains of 0.1 to 0.2% for meat samples thawed at 4°C when the relative humidity was 90%. They concluded that frozen meat samples acted as a cold surface condensor in respect to the surrounding air which cooled down below the dew point causing water to condense on the surface and be absorbed by the meat. Similar observations were reported by Kassai (1970).

Weight loss during cooking

Moisture loss during cooking can greatly influence the juiciness and texture of cooked meat (Wierbicki et al., 1957a). In frozen meat products the rate of freezing and thawing has been shown to affect cooking loss which, in turn, influences flavor, palatability, tenderness and juiciness.

Brady et al. (1942) found that the smallest total cooking losses in steaks resulted from quick freezing at -26°C and cooking from the frozen state compared with those frozen at -18°C or thawed before cooking. Footrakul (1976) reported that meat samples frozen at -44.4°C lost more weight

after cooking than the ones frozen at -17.8°C. Sebranek (1977) compared cryogenically frozen and still air frozen patties and found cooking losses to be 27.7 and 36.3% respectively.

Causey et al. (1950) reported that weight loss was greater in patties thawed prior to cooking than those cooked from the frozen state. Conversely, Smith et al. (1969) found that thawing steaks prior to cooking had no significant effect on cooking loss, however, unfrozen rib steaks sustained less cooking loss than frozen steaks. Jakobsson and Bengtsson (1973) reported a significant advantage in yield for cooking from the frozen state.

Pearson and Miller (1950) reported significant increases in cooking loss after 90 days of storage but found that rate of freezing had no effect on cooking loss percentages. Law et al. (1967) stored boneless beef steaks at -18°C and -23°C for 6 months and found significant increases in cooking loss at the warmer storage temperature.

Organoleptic quality

Opinions vary in the literature as to what effect freezing and, more specifically, freezing rate have on palatability of meat, particularly on tenderness, juiciness and flavor.

Child and Paul (1937) reported that the overall palatability of meat was unaffected by freezing treatment. Brady et al. (1942) found that overall acceptability scores indicated no difference between fast frozen (-26.1°C) and slow frozen (-17.8°C) lamb steaks, or between steaks thawed before or during cooking. Work by Ary and McLean (1946) found no appreciable differences in "eating quality" of lamb legs frozen at -7°, -12°, -18° and -23°C. Pearson and Miller (1950) reported that rate of freezing did not influence tenderness or palatability but as length of storage was increased from zero to 90 days, there was a significant increase in cooking loss, total weight loss and expressible fluid. Steaks decreased in tenderness after 60 days storage but no further changes in tenderness were detected after 90 days. In an earlier storage study, Ramsbottom (1947) concluded that frozen storage of beef steaks at -23°C or lower for as long as 7 years did not significantly affect tenderness. Dawson et al. (1959) found little difference between beef that was frozen and beef that was not frozen. Footrakul (1976) compared quality traits of ground beef frozen at -17.8°C and -44.4°C and subsequently thawed at 4.4°C and 21.1°C. Taste panel results indicated no significant differences in flavor of the meat at different freezing and thawing rates. Juiciness scores were higher for samples frozen at -17.8°C and thawed at the higher temperature.

Taste panel scores also indicated slow frozen (-17.8°C) ground beef was more tender than those frozen at -44.4°C, however, no differences were detected between fast and slow frozen samples when objectively measured by shear force. Berry et al. (1971) found that palatability characteristics were generally unaffected by either freezing temperature (-18°C or -196°C) or length of frozen storage (1, 21 and 42 days).

Other workers have shown faster freezing rates to have a favorable effect on meat palatability. Hankins and Hiner (1940) reported significant differences in beef tenderness attributable to the temperature at which the meat was frozen. They further stated that meat juiciness and cooking loss differences were also a consequence of freezing temperature. Hiner and Hankins (1946) froze 3.8 cm beef cubes in still air at -8°, -18°, -23°, -40° and -81°C and in air blast at -40°C. At -8°C water was drawn from the fibers and crystals formed between the fibers creating large ice areas. With lower temperatures, crystals became smaller and more numerous and were evenly distributed within the fiber itself. At -23°C the crystals became so numerous within the fiber that some splitting of the fiber wall occurred. At -81°C the fiber splitting was more evident and they concluded that as freezing temperature was lowered tenderness increased. Later work by Birkner and Auerbach (1960) confirmed that the size, location and expansiveness of ice crystals is a consequence

of the temperature at which freezing is accomplished. Guenther and Henrickson (1962) found that freezing rates increased steak tenderness and that steaks frozen at -18°C and below were slightly more tender than unfrozen steaks. Ryan (1966) reported results of organoleptic tests conducted on poultry frozen with liquid nitrogen and conventional freezing. Liquid nitrogen frozen poultry was superior in texture, flavor, juiciness and color. Gray (1967) claimed advantages of less dehydration and moisture loss with liquid nitrogen frozen patties. Lind et al. (1971) reported significantly higher tenderness scores for thawed lamb chops frozen in moving air at -29°C and by liquid nitrogen vapor at -107°C than for those frozen in still air at -18°C. However, when the lamb chops were cooked from the frozen state, tenderness scores and Warner-Bratzler shear values did not differ significantly for chops frozen by the three methods. Sebranek et al. (1978) found patties frozen with cryogens at -74°C were more tender and juicy than similar patties frozen in still air at -29°C.

Much of the apparent confusion concerning the effect of freezing and freezing rate on organoleptic quality of meat may be attributed to the generally vague definitions of freezing rates used. Other potential sources of variation have been suggested. Hiner and Hankins (1951) stated that the tenderizing effect of freezing varies with muscle and

with age of the animal. Another possibility was offered by Weir (1960). She stated that differences in tenderness, juiciness, flavor and overall palatability ratings of beef steaks were the result of differences in the final degree of doneness to which the meat was cooked. The conclusion that increased degree of doneness decreases beef tenderness ratings was supported by others (Cover and Hostetler, 1960; Smith et al., 1968). Thus, it appears that cooking, itself, can cause significant variation in any sensory evaluation irrespective of freezing rate, and should be precisely controlled in taste panel comparisons. Part of the difficulty also stems from the inexactions of subjective evaluation of quality.

Postmortem Properties of Meat

For some time after an animal has been killed, muscle tissues retain many of the properties they had during life. Muscle in the postmortem, prerigor state is highly extensible and remains so until all the adenosine triphosphate (ATP), the immediate source of chemical energy for muscular contraction, is broken down. In full rigor, the muscle is firm and relatively inextensible. Usually several hours elapse before rigor mortis is fully developed (Bendall, 1960).

During this prerigor period, glycogen, the carbohydrate reserve in muscle, is converted to lactic acid and as a result the pH of the muscle falls. After 24 hours postmortem,

the pH falls to a level which is normally in the range of 5.4 to 5.8. This compares with a pH of about 7.0 to 7.2 in the living tissue. The rate of fall of pH is largely dependent on postmortem tissue temperature (Bate-Smith and Bendall, 1949; Marsh and Thompson, 1958; Bendall, 1960; Cassens and Newbold, 1967).

Chilling prerigor meat

Prerigor and postrigor muscles differ in their initial reaction to cooling temperatures. If the temperature falls to below 10°C in prerigor muscle before the ATP supply is depleted, the muscle will contract. This phenomenon called cold-shortening was first described by Locker and Hagyard in 1963. Cold-shortening can be averted by rapidly freezing the muscle so that ice crystal formation is complete within 10 minutes (Voyle, 1974). However, another problem termed "thaw rigor" is then possible.

Marsh and Thompson (1957) reported that meat frozen before rigor mortis, upon thawing, had an extreme shortening of the muscle fibers (30-70%) accompanied by the exudation of large amounts of fluid. Weiner (1964) supported these results. The amount of cold-shortening and thaw rigor decrease as the period between slaughter and exposure to cold or freezing conditions is extended (Marsh and Thompson, 1958; Locker and Hagyard, 1963).

Thaw rigor in muscle frozen prerigor can be prevented by keeping the muscle at a temperature just below its freezing point for several days before allowing it to thaw. Under these conditions the chemical changes associated with the development of rigor mortis are completed while there is sufficient ice in the muscle to prevent shortening (Moran, 1930; Marsh and Thompson, 1958).

Processing advantages of ground prerigor meat

Although problems are apparent with the freezing of intact prerigor muscle, the utilization of ground prerigor meat has several advantages. Hamm reported in 1959 that grinding prerigor meat did not significantly change the post-mortem pH/time relationship. Hamm (1977) further stated that ground muscle in the prerigor state had higher water holding capacity and better fat emulsification properties than normal rigor or postrigor muscle. He concluded that prerigor sausage products had less moisture loss and less rendering out of fat when cooked.

Lin (1976) compared fresh pork sausage made from pre- and postrigor meat. Prerigor sausage had lower rancidity values after prolonged storage, higher flavor, juiciness and overall acceptance scores, and less cooking loss than post-rigor sausage.

Ice crystal formation in prerigor meat

A few researchers have shown that ice crystal formation in prerigor tissue is not characteristic of postrigor tissue.

In 1940 Ramsbottom and Koonz froze beef muscle at different times after death and investigated the nature of the ice crystals. They reported that ice formed inside the cells in prerigor muscle (6 hours after slaughter) but was outside the cells when freezing 24 hours postmortem. On freezing 35 days postmortem, the water froze in much larger extracellular crystals. Later, Love and Haraldsson (1961) showed that freezing prerigor cod muscle at any speed always resulted in intracellular freezing. Even at freezing rates beyond 100 minutes (for 0 to -5°C change) ice in the prerigor muscle formed intracellularly while postrigor fillets exhibited extracellular freezing as usual. In addition to these observations, Love (1962) noted that ice crystals formed in prerigor muscle were "rosette" shaped and not rounded as those found in normal postrigor muscle. The causes for these apparent differences in prerigor muscle have not been determined.

MATERIALS AND METHODS

Experimental Design

This study was designed to determine the effect of four freezing rates and six pattie formulations on the microstructure and quality of ground beef patties. The ground beef patties used as experimental units were formulated to be similar in weight and proximate composition (moisture, fat and protein).

Six pattie formulations were studied:

1) 0% frozen-flaked trim, 100% unfrozen trim.
2) 20% frozen-flaked trim, 80% unfrozen trim.
3) 50% frozen-flaked trim, 50% unfrozen trim.
4) 100% frozen-flaked trim, 0% unfrozen trim.
5) 100% prerigor trim.
6) 100% postrigor trim.

Each of the six pattie formulations was frozen by each of the four freezing rates. The freezing rates selected were calculated as the amount of time necessary for the internal temperature of the pattie to change from +5° to -5°C during the freezing process. The measured times for the four freezing rates were 6, 30, 80 and 100 minutes respectively. Patties frozen by these methods were also compared qualitatively to unfrozen patties.

Boneless Beef Trim Preparation

Frozen-flaked trim

Meat utilized for frozen-flaked trim was removed from 600 to 800 lb. "A" maturity steer carcasses having Choice or Good quality grades. All carcasses were held at 5°C for 24 to 72 hours postmortem prior to use. During boning, lean tissue and fat were combined in batches comprising approximately 20% fat and 80% lean tissue. The boneless beef was coarse ground through a 2.54 cm plate in a Weiler grinder (Model No. 6) in batches of 27.3 kg. The percentage of fat for each batch was approximated by an Anyl-Ray fat testing machine (Anyl-Ray Corporation). Each batch of ground beef trim was packed in plastic lined boxes and stored at -30°C for at least 48 hours before flaking for subsequent pattie formulation. Flaking was accomplished using a Butcher Boy Flaker (Model C.M.F., Lasar Mfg. Corp.). After flaking, the frozen-flaked trim (-1.6°C) was initially ground through a 0.95 cm plate into a Biro mixer-grinder. At this time the various combinations of nonfrozen and frozen-flaked trim were combined into formulations containing 0, 20, 50 and 100% frozen-flaked trim. Each trim formulation was then mixed for 60 seconds and reground (0.32 cm plate) before pattie formation.

Nonfrozen trim

Boneless beef utilized for nonfrozen trim was obtained from similar carcasses. An initial coarse grinding (2.54 cm

plate) was done before testing for fat percentage. After a second grinding (0.95 cm plate), various nonfrozen/frozen-flaked trim formulations were combined in the Biro mixer-grinder and mixed for 60 seconds before a final grind through a 0.32 cm plate.

Prerigor trim

Immediately after slaughter, the left side of a 750 lb. "A" maturity, Good grade steer carcass was hot-boned and coarse ground (2.54 cm plate) before chilling to 5°C with carbon dioxide snow in a Rietz blender. After chilling and fat analysis, the ground beef was again ground (0.95 cm plate), mixed and reground (0.32 cm plate) before it was used for pattie formulation.

Postrigor trim

The remaining right side of the carcass used for the prerigor trim was held at 5°C for 48 hours postmortem and then boned out. This boneless beef trim was treated in an identical manner to the nonfrozen trim previously discussed.

Ground Beef Pattie Formulation

After preparation of the frozen-flaked, nonfrozen, prerigor and postrigor trim, six types of patties were formu-

lated: (1) 100% nonfrozen trim, (2) 20% nonfrozen/80% frozen-flaked, (3) 50% nonfrozen/50% frozen-flaked, (4) 100% frozen-flaked trim, (5) 100% prerigor trim and, (6) 100% postrigor trim. Each formulation was mechanically formed into patties with a Hollymatic Model 500A pattie machine. The pattie machine was calibrated to form patties weighing approximately 113.5 grams with a diameter of 11.0 cm and a thickness of 1.0 cm. All patties contained approximately 20% fat. Prerigor patties were made and frozen within 3 hours postmortem. Patties were formed when trim temperature was 5°C.

Freezing Treatments

Patties prepared from each ground beef formulation were divided into five groups, four for freezing and one for unfrozen comparisons. Four freezing environments, each with a specific freezing rate (time for +5° to -5°C change) were selected. All internal pattie temperature measurements were recorded with teflon insulated copper-constantan thermocouples attached to a Honeywell temperature recording machine. All patties were weighed prior to and after freezing. Weight loss due to freezing was calculated and expressed as a percentage of prefrozen pattie weight.

After freezing, all patties were wrapped in groups of six with plastic coated freezer paper before storing (see follow-

ing) in polyethylene bags for subsequent tests and microscopic evaluation.

The unfrozen patties were covered with freezer paper and placed in sealed plastic bags until used. Quality tests and preparation for microscopic evaluation were initiated within 72 hours of pattie formulation.

6 Minute freezing rate

Formulated ground beef patties were placed on the entry end of a conveyor belt in a Kwik Freeze carbon dioxide freezing tunnel (AirCo Corporation). They were exposed to a -80°C temperature for 8 minutes as they passed through the tunnel. The internal temperature of the patties ranged from -17.8 to -20.5°C immediately after the cryogenic freezing. All patties from this freezing treatment were stored at -30°C.

30 Minute freezing rate

Formulated ground beef patties were placed in single layers on paper in wire baskets and exposed to -30°C moving air currents for 6 hours before wrapping. The blast freezer (Krack Model DT-200-DXF-HG) had an air change rate of 1.14 and an air velocity of 4040 cubic feet per minute. All patties from this freezing treatment were stored at -30°C.

80 Minute freezing rate

Formulated ground beef patties were placed in single layers on paper in wire baskets and exposed to -15°C for 6

hours in an identical blast freezer to that used for the 6 minute freezing rate. Patties used for taste panel evaluation and microstructure analysis from this freezing treatment were stored at -30°C. Patties used for all other tests from this freezing treatment were stored at their freezing temperature (-15°C).

100 Minute freezing rate

Formulated ground beef patties were placed in single layers on papered freezer shelves in an upright home-type freezer (Kenmore Coldspot). They were exposed for 6 hours to freezing temperatures fluctuating between -5° and -12°C. Patties used for taste panel evaluation and microstructure analysis from this freezing treatment were stored at -30°C. Patties used in all other tests for this freezing treatment were stored at their freezing temperature (-5° to -12°C).

Thawing Treatments

Frozen patties from 24 treatment combinations (4 freezing rates X 6 pattie formulations) were thawed under two conditions. One group of patties was thawed in a chamber held at 5°C (relative humidity 80%) while the other group was thawed at room temperature (20°C, 65% relative humidity).

Frozen patties were weighed and placed in single layers on wire baskets in their respective chambers. Thawing was

considered complete when the internal temperature of the patties reached 5°C. At this temperature, the thawed patties were reweighed for weight gain or loss, and subsequently cooked.

Cooking Treatments

All pattie formulations were grilled under the following conditions: 1) from the unfrozen state, 2) from the frozen state after the patties were frozen at the four rates, 3) after thawing at 5°C and 4) after thawing at 20°C. Grill temperature was 190.5°C and all patties were removed from the grill when an internal pattie temperature of 60°C was recorded. Cooking loss was the calculated weight loss during cooking and expressed as percentage of precooked pattie weight.

Organoleptic Evaluation

Sensory quality was determined by a nontrained taste panel comprised of 12 to 18 I.S.U. staff members and graduate students. They evaluated four quality attributes: flavor, texture, juiciness and overall acceptability by using a nine-point hedonic scale (Figure 1) with 9 representing the most desirable and 1 representing the least desirable.

Patties from all treatment combinations were grilled as previously described from the unfrozen and frozen state. No thawed patties were grilled for organoleptic evaluation.

Judge _____

Date _____

FLAVOR		TEXTURE		JUICINESS		OVERALL	
Extremely desirable	9	Extremely tender	9	Extremely juicy	9	Like extremely	9
Very desirable	8	Very tender	8	Very juicy	8	Like very much	8
Moderately desirable	7	Moderately tender	7	Moderately juicy	7	Like moderately	7
Slightly desirable	6	Slightly tender	6	Slightly juicy	6	Like slightly	6
Neither desirable nor undesirable	5	Neither tender nor tough	5	Neither juicy nor dry	5	Neither like nor dislike	5
Slightly undesirable	4	Slightly tough	4	Slightly dry	4	Dislike slightly	4
Moderately undesirable	3	Moderately tough	3	Moderately dry	3	Dislike moderately	3
Very undesirable	2	Very tough	2	Very dry	2	Dislike very much	2
Extremely undesirable	1	Extremely tough	1	Extremely dry	1	Dislike extremely	1

SAMPLE	FLAVOR	TEXTURE	JUICINESS	OVERALL

Comments:

Figure 1. Sensory evaluation of hamburger

Analytical Methods

Proximate analysis

Three randomly selected, unfrozen patties from each formulation were analyzed for moisture and fat content according to procedures described by AOAC (1975). Also, different areas of an individual frozen pattie were sampled and similarly analyzed for comparison in an attempt to check for sampling differences within a pattie. Three patties from each treatment combination were used. Six random core samples (10 grams total) taken from the perimeter of each frozen pattie were compared to core samples taken from the center of the same pattie.

pH

The pH of the unfrozen and frozen patties was measured using a Corning digital pH meter. A 10 gram sample was added to 90 ml of distilled water and blended for one minute before measuring.

Water holding capacity

The water holding capacity of the unfrozen pattie formulations was measured according to a basic press method originally described by Grau and Hamm (1953) and later by Briskey et al. (1960). Approximately 0.3 grams of the ground beef pattie sample was placed between two plexiglass plates

that were pressed together by a 20,684 kPa pressure for 3 minutes. The resulting two areas on the filter paper, total juice or moisture area and meat area, were measured with a compensating planimeter. Water holding capacity was expressed as the ratio of total juice or moisture area to meat area.

The percentage of free water in the unfrozen samples was calculated on an absolute basis as follows (Briskey et al., 1960:

$$\% \text{ free water} = \frac{(\text{total area-meat area})(44.07)}{\text{total water content (mg)}} \times 100$$

Reflectance value and color

Reflectance values of unfrozen and all frozen patties were measured with a Photovolt Photoelectric Reflection Meter (Model 670). This machine was designed to measure the diffuse reflection of surfaces as an indication of lightness or darkness of a particular color. The higher the reflectance value, the lighter the color of the pattie. Color photographs were also taken of the six pattie formulations before and after freezing at the four freezing rates.

DNA content of drip fluid

Frozen patties from all treatment combinations were placed in 15 cm diameter funnels and allowed to collapse into

the funnels during an 8 hour thaw period at 20°C. Weight loss of the pattie during this thaw period was considered drip loss and expressed as a percentage of frozen pattie weight. Drip fluid collected in jars below the funnels was analyzed for DNA content. The modified Schmidt-Thannhauser (1945) method, as recommended by Munro and Fleck (1966) and further modified by Trenkle et al. (1978) was used for the determination of DNA in the drip fluid samples. The exact procedure for this analysis is described in Appendix B.

Protein content of drip fluid

Drip was collected from frozen patties as previously described for DNA determination. Protein concentration of the drip fluid was determined by the Biuret method as described by AOAC (1965). The exact procedure for this analysis is outlined in Appendix B.

Texture

The patties for the Warner-Bratzler shear were cooked in the same manner and to the same internal temperature as previously described (see page 42). The patties were allowed to cool to room temperature before core samples were removed for shearing. Three 2.54 cm cores were removed from each of five patties from each treatment combination. All 15 cores were then sheared once by the Warner-Bratzler shearing device (Bratzler, 1949).

Preparation of Pattie Samples for Light Microscopy (LM) Evaluation

Unfrozen samples

Unfrozen patties were stored at 5°C prior to preparation for light microscopy. One core sample (1.27 cm diameter) was removed from the center of each of five randomly selected patties from each pattie formulation. Each core was frozen by immersion in a liquid nitrogen (LN_2) bath (-195°C) for one minute. Frozen core samples were then cross-sectioned into 0.6 cm pieces before mounting on a freezing microtome chuck using OCT compound (Fisher Scientific Co). Frozen, mounted samples were allowed to temper to -25°C prior to tissue sectioning. All samples were sectioned at a thickness of 18 µm on a Tissue-Tek II Cryostat. The frozen samples were shaved down at least 200 µm before initiating collection of the tissue sections. Frozen sections were sliced and picked up on a warm glass slide. At least 50 representative sections from each treatment were collected from a minimum of ten frozen pattie samples. Tissue sections were allowed to air dry for one hour and then stained with oil red 0 and hematoxylin (Humason, 1972) as described in Appendix B.

Light micrographs of unfrozen pattie samples from each formulation were taken on Bausch and Lomb Balplan light microscope. All photomicrographs were taken at the same magnifi-

cation for comparison on Polaroid type 665 P/N black and white film.

Frozen samples

A mechanical boring device (Kastner and Henrickson, 1969) was used to remove core samples from frozen patties. One core sample (1.27 cm diameter) was removed from the center of each of five randomly selected patties from each treatment. Each frozen core was mounted, sectioned, stained and photographed in an identical manner to the procedure previously described for LM examination of unfrozen pattie samples.

Preparation of Pattie Samples for Scanning Electron Microscopy (SEM) Evaluation

Unfrozen samples

Core samples from each unfrozen pattie formulation were removed and frozen in an identical manner as unfrozen samples prepared for LM evaluation. Once frozen, cores were cryo-fractured by placing the sample between two chilled, flat metal surfaces and striking the upper surface with a mallet. Sample pieces of less than 2 cubic millimeters were placed in prechilled glass vials and buried in carbon dioxide snow to prevent recrystallization. These vials were then placed in a Virtis laboratory freeze-drier prechilled to a chamber temperature of -40°C. After 48 hours of freeze-drying, sample vials

were removed, capped and stored in a vacuum desiccator until final SEM preparation.

Samples used for SEM evaluation were mounted on brass stubs (9 mm diameter) with silver conducting paint (Ladd, #60730). The samples were transferred to a Varian VE-30 High Vacuum Evaporator. A 25 cm piece of 0.02 cm diameter gold wire was coiled and placed in a tungsten filament basket. Carbon electrodes were also prepared and inserted into the evaporator. Under a high vacuum (2.5×10^{-5} Torr) a current was applied first to the carbon electrodes and then the gold. As current was increased to the electrodes, carbon and gold were evaporated in the chamber and deposited on the samples. During evaporation, the specimen stage was rotated to ensure even distribution of evaporated metals on the sample surfaces.

After coating, samples were ready for observation with the SEM. SEM micrographs of pattie samples from each unfrozen formulation were taken using a JEOL JSM-35 scanning electron microscope. Samples were all observed using a primary electron beam with an accelerating voltage of 15 kilovolts and an 80 μA beam current. A 100 μm diameter objective aperture was used. Specimen orientation and lens currents were adjusted as necessary to give optimum performance and to produce micrographs of high image quality. The recording cathode ray tube was maintained at 2500 lines per frame. All SEM micrographs were taken on Polaroid type 665 P/N film.

Frozen samples

Core samples were removed from frozen patties of each treatment combination identically to those prepared for LM evaluation. Once removed, frozen cores were immersed in a LN_2 bath for one minute and cryofractured, freeze-dried, metal coated, mounted and photographed with the SEM using identical procedures previously described for SEM preparation and examination of unfrozen samples.

Unfrozen, cooked samples

Unfrozen patties from three trim formulations (100% non-frozen, 100% frozen-flaked and 100% prerigor) were grilled and sampled as previously described (see texture, page 46). Cooked core samples were immersed in a LN_2 bath for one minute, then cryofractured, freeze-dried, metal coated, mounted and photographed with the SEM using identical procedures previously described for SEM examination of unfrozen and frozen samples.

Frozen, cooked samples

Patties from three trim formulations (100% nonfrozen, 100% frozen-flaked and 100% prerigor) and frozen at four freezing rates (6, 30, 80 and 100 minutes) were grilled, sampled and prepared for SEM evaluation in the same manner as the unfrozen cooked patties.

Statistical Analysis

The data were analyzed utilizing the Statistical Analysis System of Barr et al. (1976) and by methods described by Snedecor and Cochran (1967). The analysis of variance and Duncan's Multiple Range Test were used to determine the significance of variability of the means for all traits measured.

RESULTS AND DISCUSSION

Proximate Analysis and Sampling

An analysis of variance showed no significant differences among the six formulations (Figure 2) used in the study for moisture and fat percentages indicating the uniformity of the patties used as experimental units. Moisture values ranged from 60.62 to 63.20% while fat values were from 17.68 to 21.61%. Within a frozen pattie, no significant differences were found when comparing perimeter samples to those taken from the center of the pattie. This indicated that moisture loss due to freezing apparently was consistent over the entire pattie surface and that selection of a representative sample area for subsequent microstructure analysis was not critical.

Effects of Freezing Rate and Formulation on Color, pH, Water Holding Capacity and Freezing Shrink

Color

Examples of color differences due to formulation and freezing rate are shown in Figure 2. Photographs of the unfrozen patties were taken immediately after processing while photographs of the frozen patties were taken after one month of storage at their respective freezing temperature. Corresponding color reflectance values are given in Table 1.

Figure 2. Ground beef patties representing each treatment combination

Number denotes formulation:

1) 0% Frozen-flaked trim, 100% nonfrozen trim
2) 20% Frozen-flaked trim, 80% nonfrozen trim
3) 50% Frozen-flaked trim, 50% nonfrozen trim
4) 100% Frozen-flaked trim, 0% nonfrozen trim
5) 100% Prerigor trim
6) 100% Postrigor trim

Letter denotes freezing rate:

A) Unfrozen
B) Freezing rate: 6 minutes
C) Freezing rate: 30 minutes
D) Freezing rate: 80 minutes
E) Freezing rate: 100 minutes

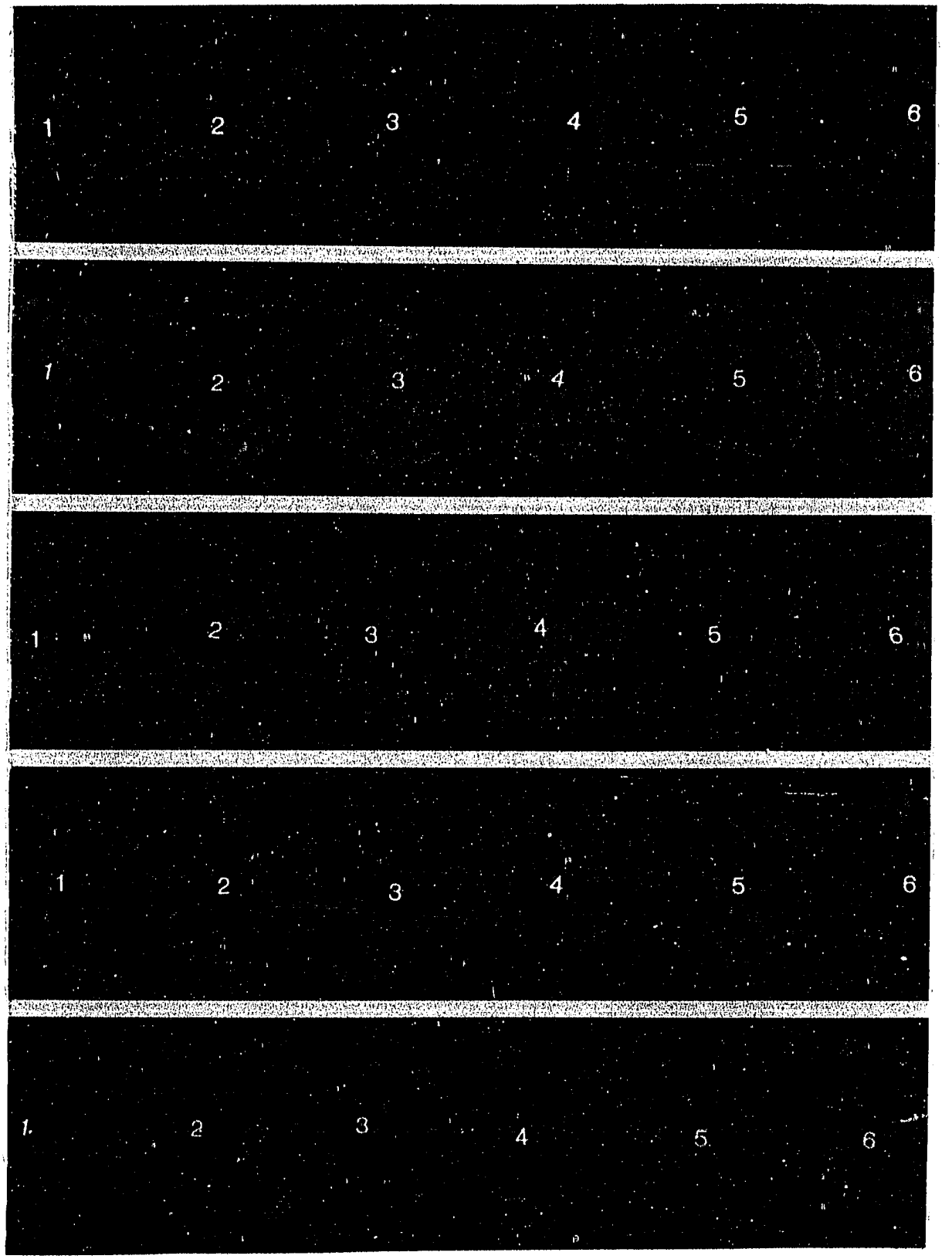
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Table 1. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for color reflectance values[4]

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)					Overall treatment means
	Unfrozen	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	22.60 1.83	32.40 0.53	32.67 1.61	28.00 0.50	19.00 0.50	26.93[c,d] 5.67
20% Frozen trim	22.80 1.47	33.33 1.53	32.67 0.76	27.50 0.50	22.30 0.17	27.72[c] 4.92
50% Frozen trim	25.77 0.86	35.00 0.87	34.00 1.00	29.67 0.76	23.17 1.61	29.52[b] 4.82
100% Frozen trim	27.50 0.50	42.17 1.76	41.33 0.29	32.50 0.50	25.00 0.87	33.70[a] 7.30
Prerigor trim	18.87 1.03	31.33 0.29	32.33 0.58	25.33 2.08	21.83 1.26	25.94[e] 5.52
Postrigor trim	21.83 0.76	32.17 1.04	31.33 1.53	27.00 0.87	19.23 1.75	26.31[d,e] 5.38
Overall treatment means	23.23[c] 3.02	34.40[a] 3.88	34.06[a] 3.56	28.33[b] 2.49	21.76[d] 2.39	

[1]n=3 observations per cell.
[2]Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).
[3]Standard deviations are listed below each mean.
[4]Larger values indicate lighter colors.
[5]Frozen trim denotes frozen-flaked trim.

Color reflectance values increased as percentage of frozen-flaked trim was increased, with the 100% frozen-flaked formulation recording the highest value among all formulations and within each freezing rate. Prerigor patties were darker than postrigor patties before freezing, but color differences were much less detectable after freezing. Slower freezing rates produced a darker color which is in agreement with the results reported by Guenther and Henrickson (1962), Jakobsson and Bengtsson (1973), and Sebranek et al. (1978). This may be due to larger ice crystal size caused by slower freezing rates (Menz and Luyet, 1961). Discoloration of the 100% frozen-flaked pattie, frozen at the 100 minute freezing rate (E-4), may indicate potential problems for patties with this formulation and stored at higher temperatures.

pH

With the exception of prerigor patties (Table 2), no significant differences in pH were found between patties measured in the frozen or thawed states. This indicates that the freezing and thawing process apparently does not alter pH (Deatherage and Hamm, 1960). Also, pH values of patties compared for different freezing rates were essentially the same.

As mentioned, only prerigor patties had a significant pH change during thawing. An example is shown in Table 2. At

Table 2. A comparison of pH means[1] and standard deviations[2] for thawed or frozen patties from two freezing rates

Formulation[3]	Freezing rate (Time for +5°C to -5°C Change) 6 min.	
	Frozen	Thawed
0% Frozen trim	5.68[a] 0.00	5.74[a] 0.08
20% Frozen trim	5.65[a] 0.04	5.73[a] 0.04
50% Frozen trim	5.67[a] 0.04	5.71[a] 0.02
100% Frozen trim	5.62[a] 0.01	5.60[a] 0.03
Prerigor trim	5.83[b] 0.06	5.37[b] 0.02
Postrigor trim	5.67[a] 0.04	5.62[a] 0.01

[1]Means in the same column with the same superscripts are not significantly different ($p<0.05$).

[2]Standard deviations are listed below each mean.

[3]Frozen trim denotes frozen-flaked trim.

the 6 minute freezing rate, the prerigor patties were significantly higher (5.83) than all other formulations. After thawing, the pH of the prerigor patties dropped to 5.37 and was significantly lower than all other formulations. Evidently, the high pH associated with prerigor muscle (Bendall, 1960) was preserved with freezing, but dropped significantly during thawing as glycolysis accelerated and rigor mortis developed (Marsh and Thompson, 1958).

Water holding capacity

Water holding capacity (WHC) of the six pattie formulations was measured prior to freezing (Table 3). WHC increased significantly with greater proportions of frozen-flaked trim as evidenced by lower ratios (Table 3) and lower percentages of free water. This was particularly evident when comparing the 100% frozen-flaked formulation with those having no frozen-flaked trim. Flaking apparently increases WHC in a similar fashion to grinding (Hamm, 1959, 1977). The process results in exposing more polar groups for binding with the water molecule.

Prerigor patties also showed significantly greater water holding capacity than postrigor patties. The greater water retention of this formulation is associated with the high pH of prerigor meat (Table 2) and is in agreement with the data reported by Swift and Berman (1959), Hamm (1960) and McClain and Mullins (1969).

Table 3. Means[1,2,3] for water holding capacity[4] and percentage free water[5] of nonfrozen, noncooked patties

Formulation[6]	Water holding capacity	% Free water
0% Frozen trim	2.81[a] 0.21	85.54[a] 8.19
20% Frozen trim	2.27[b] 0.17	67.84[b] 4.88
50% Frozen trim	2.06[b,c] 0.13	59.29[b,c] 9.98
100% Frozen trim	1.76[c] 0.13	47.67[c] 7.02
Prerigor trim	2.13[b] 0.08	58.73[b,c] 6.77
Postrigor trim	2.75[a] 0.33	69.60[b] 9.60

[1]n=3 observations per cell.

[2]Standard deviations are listed below each mean.

[3]Treatment means in a column with the same superscripts are not significantly different ($p<0.05$).

[4]Using press method and expressed as a ratio of total moisture area/meat area. Higher value indicates lower WHC.

[5]Percent free water =

$$\frac{(\text{moisture area} - \text{meat area})(44.07)}{\text{sample water content (mg)}} \times 100$$

[6]Frozen trim denotes frozen-flaked trim.

Freezing shrink

Freezing shrink values shown in Table 4 indicate no differences among the six formulations, however, freezing rate had a significant effect on freezing shrink. The superiority of faster freezing rates in reducing evaporation during the freezing process is clearly evident and substantial. Any frozen food processor should be genuinely concerned with weight loss during freezing. Even a small decrease in shrink can mean significantly greater profits when dealing with large volumes of product.

Effects of Freezing Rate and Formulation on Thawing Characteristics

Weight gain

Two groups of patties, each representing all treatment combinations were thawed to an internal temperature of 5°C in temperature controlled rooms set at 5° or 20°C (Tables 5 and 6). Both thaw groups experienced weight gain during the thawing period which ranged from 75 to 90 minutes. This agrees with thawing results recorded by several workers (Paul and Child, 1937; Dunker and Hankins, 1953; Kassai, 1970). Patties thawed at room temperature (20°C) had a significantly greater weight gain as a group (1.06%) than patties thawed at the cooler temperature (0.89%). This is probably due to the greater temperature differential between pattie surface and

Table 4. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for freezing shrink percentage[4]

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)				Overall treatment means
	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	0.48 0.08	1.88 0.23	2.96 0.15	4.10 1.43	2.36[a] 1.53
20% Frozen trim	0.52 0.15	2.00 0.25	3.20 0.12	4.88 1.43	2.65[a] 1.77
50% Frozen trim	0.38 0.16	2.06 0.21	3.36 0.38	4.72 0.48	2.63[a] 1.67
100% Frozen trim	0.40 0.14	1.00 0.14	2.92 0.39	5.10 1.53	2.36[a] 2.02
Prerigor trim	0.74 0.15	1.32 0.16	2.74 0.11	4.26 0.91	2.27[a] 1.46
Postrigor trim	0.64 0.15	1.40 0.16	2.78 0.16	4.84 0.15	2.42[a] 1.64
Overall treatment means	0.53[d] 0.18	1.61[c] 0.44	2.99[b] 0.32	4.65[a] 1.08	

[1] n=5 observations per cell.

[2] Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3] Standard deviations are listed below each mean.

[4] Calculated weight loss as a percentage of initial pattie weight.

[5] Frozen trim denotes frozen-flaked trim.

surrounding air temperature and high relative humidity in the 20°C chamber resulting in greater water uptake.

Weight gain during thawing also was influenced by formulation (Tables 5 and 6). Thaw gain on patties made from 100% frozen-flaked trim was significantly higher than the 0% frozen-flaked formulation for both groups. Prerigor patties also responded in a similar manner at their respective thaw temperatures. As previously mentioned, these two formulations also had the highest water holding capacity values (Table 3) prior to freezing. The ability of the pattie to bind water may, in some way, affect the amount of moisture gain on the pattie surface during thawing.

A comparison of freezing rates (Tables 5 and 6) indicates greater thaw gains on patties frozen at faster freezing rates. Since faster freezing rates also reduce moisture loss during freezing (Table 4), the significant ($p<0.01$) and negative ($r=-0.48$) correlation between freezing shrink and thaw gain (Table 19) suggests that less shrink during freezing may directly increase weight gain during thawing.

Regarding thaw weight gain, it appears that warmer thaw temperatures can significantly affect weight gain, although other factors also apparently influence moisture gain during thawing. These factors seem linked to the ability of the pattie formulation to retain water, either by greater WHC

Table 5. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for percentage weight gain[4] after thawing at 5°C

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)				Overall treatment means
	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	1.03 0.07	1.02 0.20	0.52 0.08	0.62 0.13	0.80^{c} 0.26
20% Frozen trim	1.12 0.12	1.03 0.05	0.64 0.13	0.64 0.12	$0.86^{b,c}$ 0.25
50% Frozen trim	1.09 0.14	1.00 0.10	0.82 0.12	0.82 0.23	$0.93^{a,b}$ 0.18
100% Frozen trim	1.25 0.17	1.17 0.11	0.65 0.04	0.82 0.16	0.97^{a} 0.28
Prerigor trim	1.13 0.25	1.01 0.10	0.79 0.13	0.66 0.18	$0.90^{a,b}$ 0.25
Postrigor trim	0.88 0.17	1.08 0.16	0.69 0.11	0.75 0.09	$0.85^{b,c}$ 0.20
Overall treatment means	1.08^{a} 0.19	1.05^{a} 0.13	0.69^{b} 0.14	0.72^{b} 0.17	0.89 0.24

[1] n=5 observations per cell.

[2] Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3] Standard deviations are listed below each mean.

[4] Calculated weight gain as a percentage of frozen pattie weight.

[5] Frozen trim denotes frozen-flaked trim.

Table 6. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for percentage weight gain[4] after thawing at 20°C

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)				Overall treatment means
	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	0.96 0.15	1.13 0.15	0.88 0.16	0.55 0.22	0.88[c] 0.27
20% Frozen trim	0.93 0.08	1.00 0.07	1.16 0.24	0.72 0.19	0.95[c] 0.22
50% Frozen trim	0.96 0.14	1.04 0.13	1.13 0.05	0.75 0.20	0.97[c] 0.19
100% Frozen trim	1.16 0.11	1.18 0.13	1.10 0.28	0.95 0.14	1.10[b] 0.19
Prerigor trim	1.44 0.16	1.23 0.25	1.03 0.12	1.23 0.08	1.23[a] 0.21
Postrigor trim	1.34 0.18	1.34 0.13	0.94 0.01	1.19 0.12	1.20[a] 0.20
Overall treatment means	1.13[a] 0.24	1.15[a] 0.18	1.04[b] 0.19	0.90[c] 0.30	1.06 0.25

[1] n=5 observations per cell.

[2] Treatment means in a column or row with the same superscripts are not significantly different (p 0.05).

[3] Standard deviations are listed below each mean.

[4] Calculated weight gain as a percentage of frozen pattie weight.

[5] Frozen trim denotes frozen-flaked trim.

(as with flaking) or by decreasing freezing shrink as with faster freezing rates.

Drip loss

When patties were allowed to thaw for an extended period of 8 hours (at 20°C) a weight loss occurred (Table 7 and Figure 3). Drip fluid was collected as the rigid, frozen patties were allowed to collapse into funnels upon thawing. Weight loss during this prolonged thaw period was considered drip loss. The drip fluid was analyzed for DNA and protein (Tables 8 and 9) as a possible measure of freezing damage (Love, 1966).

Drip loss (Table 7 and Figure 3) significantly ($p<0.05$) increased as amount of frozen-flaked trim in the formulation increased. No significant differences were shown, however, between the 0% and 20% frozen-flaked formulations. At the 50% and 100% levels, drip losses (7.72 and 10.36% respectively) were both significantly ($p<0.05$) greater than either the 0% or 20% formulations. Since WHC increased and percentage free water decreased with greater proportions of frozen-flaked trim (Table 3), this significant increase in drip loss was unexpected. Also, the fact that freezing shrink was not different among the formulations (Table 4), focuses the increased drip loss on the physical nature of the patties. Flaking reduces fiber particle size to a much

Table 7. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for percentage drip loss[4]

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)				Overall treatment means
	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	6.38 0.76	4.65 1.21	5.15 0.78	5.51 0.52	5.42[c] 0.98
20% Frozen trim	6.06 0.44	5.36 1.10	5.54 0.69	4.84 0.26	5.45[c] 0.75
50% Frozen trim	8.45 0.63	7.29 0.48	8.47 0.73	6.68 0.78	7.72[b] 0.98
100% Frozen trim	12.05 0.52	10.79 0.95	9.65 0.57	8.97 2.64	10.36[a] 1.74
Prerigor trim	13.45 0.29	12.64 1.72	11.58 0.74	5.32 3.98	10.75[a] 3.84
Postrigor trim	8.61 0.30	8.19 0.19	10.21 0.58	4.14 1.74	7.79[b] 2.47
Overall treatment means	9.17[a] 2.85	8.15[b] 3.04	8.43[a,b] 2.51	5.91[c] 2.40	

[1]n=3 observations per cell.

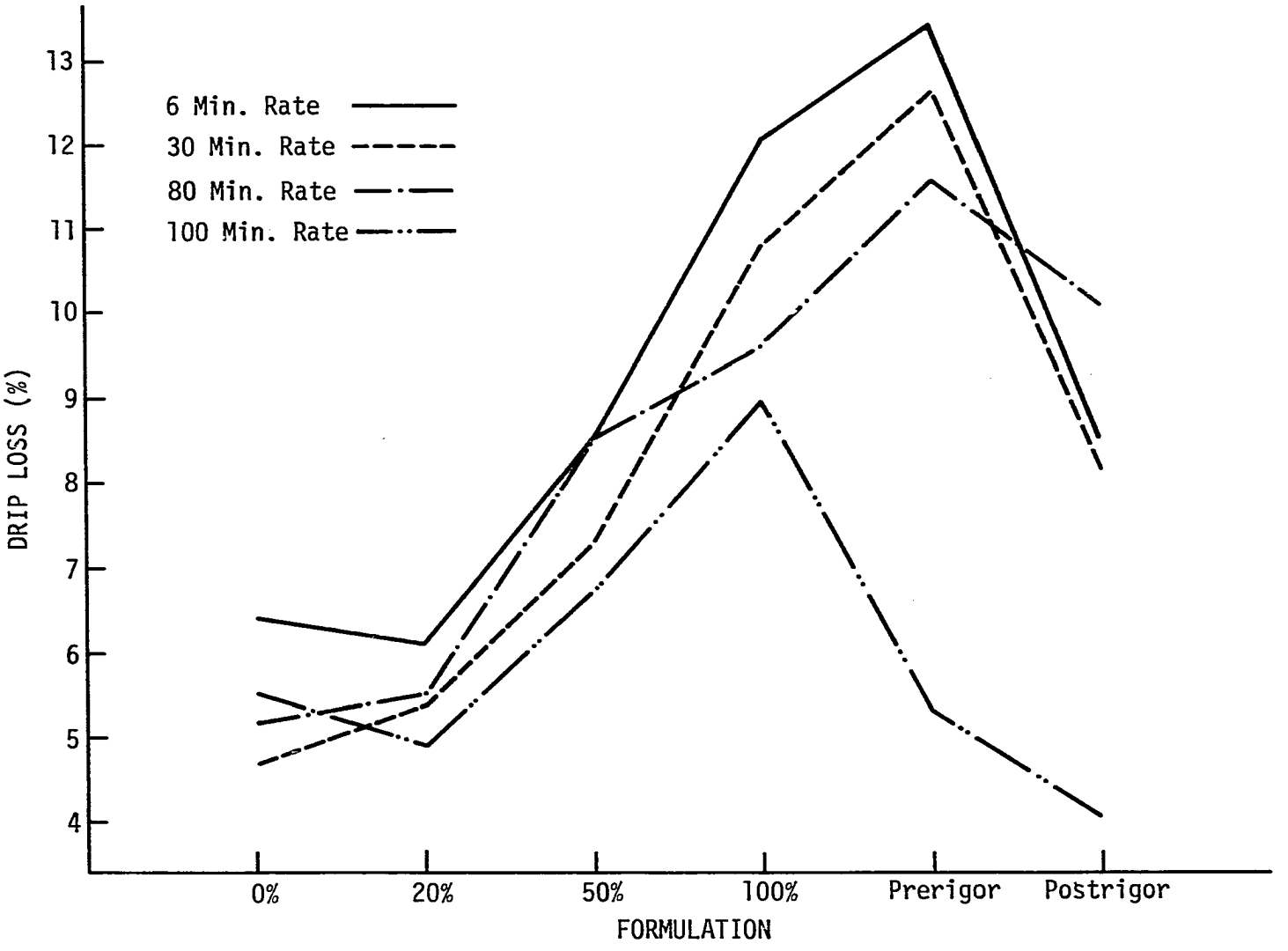
[2]Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3]Standard deviations are listed below each mean.

[4]Calculated weight loss as a percentage of frozen pattie weight. Thaw period was 8 hours.

[5]Frozen trim denotes frozen-flaked trim.

Figure 3. Effect of pattie formulation and freezing rate on percentage drip loss. Formulation percentages indicate amount of frozen-flaked trim


6 Min. Rate
30 Min. Rate
80 Min. Rate
100 Min. Rate
DRIP LOSS (%)
13
12
11
10
9
8
7
6
5
4
0%
20%
50%
100%
Prerigor
Postrigor
FORMULATION

greater extent than grinding. This was apparent by the somewhat "crumbly" nature of patties containing 50 or 100% frozen-flaked trim. Increasing surface area by flaking increases the susceptibility of the thawed meat to drip (Sair and Cook, 1938; Ramsbottom and Koonz, 1939; Penny, 1974). Therefore, increased surface area due to flaking and the subsequent crumbling of the pattie made of 50 or 100% flaked trim, during the prolonged thawing period apparently overcome the WHC advantage caused by flaking. Prerigor patties sustained drip losses equal to those of the 100% frozen-flaked patties, although this was attributed to other factors. Apparently prerigor patties when thawed experienced thaw rigor accompanied by a drop in pH (Table 2) and exudation of large amounts of fluid (Marsh and Thompson, 1957). Drip fluid from these patties appeared much more viscous and cloudy. Evidently the extreme and rapid shortening of the muscle fibers during thaw rigor forces more particulate matter out with the exudate.

Freezing rate also affected drip loss (Table 7) as shown by the significantly ($p<0.05$) lower value (5.91%) for the slowest freezing rate. However, when comparing these values it must be pointed out that the slower freezing rates also experienced greater moisture loss during freezing (Table 4). When combining freezing shrink and drip loss percentages (Tables 4 and 7) for each freezing rate, it becomes apparent

that total weight loss is similar, averaging very close to 10.4% for each rate. The significant ($p<0.01$) and negative ($r=-0.43$) correlation (Table 19) between drip loss and freezing shrink support this observation.

DNA content of drip fluid

Values for DNA content of the drip, expressed as mg DNA/ml drip, are given in Table 8. Trenkle et al. (1978) found beef muscle from 500 kg steers to contain about 0.34 mg DNA per gram of tissue. Therefore, patties used in this study (113.5 g wt.) would be expected to contain about 40 mg total DNA. Concentration values listed (Table 8) ranged from 0.069 to 0.191 mg DNA per ml of drip. Although it appears that DNA concentration per ml significantly increases with slower freezing rates, no meaningful trends were obvious when the total DNA content of the entire drip volume was considered (Table 20, Appendix A). Similar results were observed when comparing total DNA by formulation. Thus, it appears that using DNA concentration of the drip fluid as a measure of cellular disruption due to freezing, is somewhat questionable for ground, frozen products.

Protein content of drip fluid

Protein concentration (mg/ml) of the drip was analyzed by the Buiret method (AOAC, 1965) and the results are shown in Table 9. No significant trends were apparent when

Table 8. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for DNA content[4] of pattie drip

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)				Overall treatment means
	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	0.113 0.019	0.100 0.012	0.123 0.030	0.159 0.010	0.124[b] 0.028
20% Frozen trim	0.089 0.015	0.077 0.003	0.107 0.014	0.078 0.014	0.088[d] 0.016
50% Frozen trim	0.069 0.006	0.091 0.004	0.100 0.012	0.086 0.007	0.087[d] 0.013
100% Frozen trim	0.080 0.007	0.083 0.007	0.092 0.012	0.094 0.003	0.087[d] 0.009
Prerigor trim	0.070 0.005	0.079 0.003	0.081 0.009	0.191 0.008	0.105[c] 0.052
Postrigor trim	0.119 0.006	0.145 0.018	0.145 0.012	0.149 0.007	0.139[a] 0.016
Overall treatment means	0.090[c] 0.022	0.096[c] 0.025	0.108[b] 0.025	0.126[a] 0.044	

[1]n=3 observations per cell.

[2]Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3]Standard deviations are listed below each mean.

[4]Expressed as mg DNA/ml of pattie drip.

[5]Frozen trim denotes frozen-flaked trim.

Table 9. Individual cell means[1],[3] main effect treatment means[2] and standard deviations[3] for protein content[4] of pattie drip

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)				Overall treatment means
	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	363.75 19.45	342.50 24.75	380.00 0.00	330.00 0.00	354.06[b,c] 23.75
20% Frozen trim	372.50 10.61	319.25 15.20	337.50 24.75	326.50 2.12	338.94[c,d] 24.79
50% Frozen trim	325.75 6.01	336.25 37.12	332.50 3.54	317.50 3.53	328.00[d] 16.24
100% Frozen trim	396.25 5.30	275.00 35.36	310.00 7.07	328.75 15.91	327.50[d] 49.52
Prerigor trim	387.50 9.19	375.00 35.35	372.50 24.75	368.75 8.84	375.94[a] 18.60
Postrigor trim	362.75 29.34	367.50 17.68	370.25 0.35	350.00 7.07	362.63[a,b] 15.61
Overall treatment means	368.08[a] 26.21	335.92[b] 40.62	350.46[b] 28.67	336.92[b] 18.99	

[1]n=2 observations per cell.

[2]Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3]Standard deviations are listed below each mean.

[4]Determined by Biuret method and expressed as mg protein/ml of pattie drip.

[5]Frozen trim denotes frozen-flaked trim.

comparing values by formulation, although protein values for the three formulations containing frozen trim were significantly ($p<0.05$) lower than the 0% or postrigor formulations which had no frozen-flaked trim. Prerigor protein concentration, although higher (375.94), was not significantly different from the postrigor value (362.63).

Concentration of drip protein at the 6 minute freezing rate was significantly ($p<0.05$) greater than the three slower rates which had similar averages. Total protein in the drip was less as volume of drip fluid decreased, but differences were not considered significant.

Total protein in the whole pattie averages about 18% or 20.4 g after subtracting moisture and fat percentages (see page 52). Drip loss (Table 7) ranged from 10.4 g at the 6 minute freezing rate to 6.7 g at the 100 minute rate. It is interesting to note that approximately 2 to 4 grams of protein are found in the total drip volume which is 10 to 20% of the total protein in the pattie. Certainly a prolonged thaw period such as the one used for this drip analysis is uncommon, however, it does point out the potential nutritive loss associated with poor thawing techniques.

Effects of Freezing Rate and Formulation on Cooking Characteristics

In this phase of the study, patties representing all treatment combinations were grilled under the following conditions: 1) from the unfrozen state, 2) from the frozen state, 3) after thawing at 5°C, and 4) after thawing at 20°C. Cooking loss percentages for each of these cooking treatments are given in Tables 10-12.

Cooking from unfrozen and frozen states

Overall treatment means for cook loss of the frozen patties (Table 10) indicate a significant advantage for patties frozen at faster rates. No differences were apparent for cook loss between patties frozen at 6 or 30 minute rates, however, significantly greater cook losses occurred at the slower two rates. It is interesting to note that the patties frozen at the 100 minute rate, over all formulations, averaged nearly a 25% weight loss during the cooking process. This was significantly higher than all other freezing rates. All patties frozen at 6 and 30 minute rates had significantly lower cook losses than the unfrozen patties (Table 10). This indicates a definite advantage for cooking from the frozen state when patties are frozen by faster methods. Freezing rates of greater than 30 minutes seem to offer no advantage over cooking from the unfrozen state. These results support

Table 10. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for percentage cooking loss[4] for patties cooked nonfrozen and frozen

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)					Overall treatment means
	Unfrozen	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	25.24 0.21	18.66 1.48	23.23 1.46	23.57 2.76	23.69 2.26	22.88[a] 2.82
20% Frozen trim	22.69 0.57	20.52 0.85	21.79 0.76	24.06 1.82	29.62 1.03	23.74[a] 3.37
50% Frozen trim	21.73 0.74	20.36 1.46	20.18 2.15	22.98 0.80	28.51 1.44	22.75[a] 3.37
100% Frozen trim	17.29 1.11	16.30 2.46	15.87 1.55	17.66 1.08	20.07 3.08	17.44[b] 2.37
Prerigor trim	15.90 1.92	15.35 2.19	12.88 1.73	17.16 1.33	19.02 1.66	16.06[c] 2.64
Postrigor trim	21.64 2.02	20.23 2.37	21.39 2.50	25.04 1.68	25.34 2.93	22.73[a] 3.00
Overall treatment means	20.75[c] 3.45	18.57[d] 2.71	19.22[d] 4.05	21.75[b] 3.54	24.37[a] 4.48	

[1]n=5 observations per cell.
[2]Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).
[3]Standard deviations are listed below each mean.
[4]Calculated weight loss as a percentage of precooked pattie weight.
[5]Frozen trim denotes frozen-flaked trim.

data reported by Jakobsson and Bengtsson (1973) and Sebranek et al. (1978).

Increasing the amount of frozen-flaked trim (Table 10) from zero to 20 or 50%, over all freezing rates, had no significant effect on cook loss. However, with a 100% frozen-flaked formulation there was a significant ($p<0.05$) decrease in cook loss. The significant trend toward lower cook loss with greater proportions of frozen-flaked trim is apparent in Figure 4.

Prerigor patties also had a significantly ($p<0.05$) lower cook loss (Table 10 and Figure 4) than either the 0% frozen-flaked trim or postrigor formulations. It appears that during a very rapid thaw (grilling at 190.5°C), the ability of these two pattie formulations to preserve their high WHC (Table 3) is not affected, and results in a significantly lower cook loss.

Cooking after thawing at 5° or 20°C

Tables 11 and 12 compare cooking loss percentages for patties cooked after thawing. Two groups of patties representing all freezing rate/formulation combinations were thawed in a 5° or 20°C chamber to an internal temperature of 5°C. As patties within each thaw group reached this end-point temperature, they were immediately grilled to an internal temperature of 60°C. Percentage cook loss was calculated and values are shown in Tables 11 and 12.

Figure 4. Effect of pattie formulation and freezing rate on percentage cooking loss. Formulation percentages indicate amount of frozen-flaked trim. Unfrozen line refers to all patties not frozen after formulation

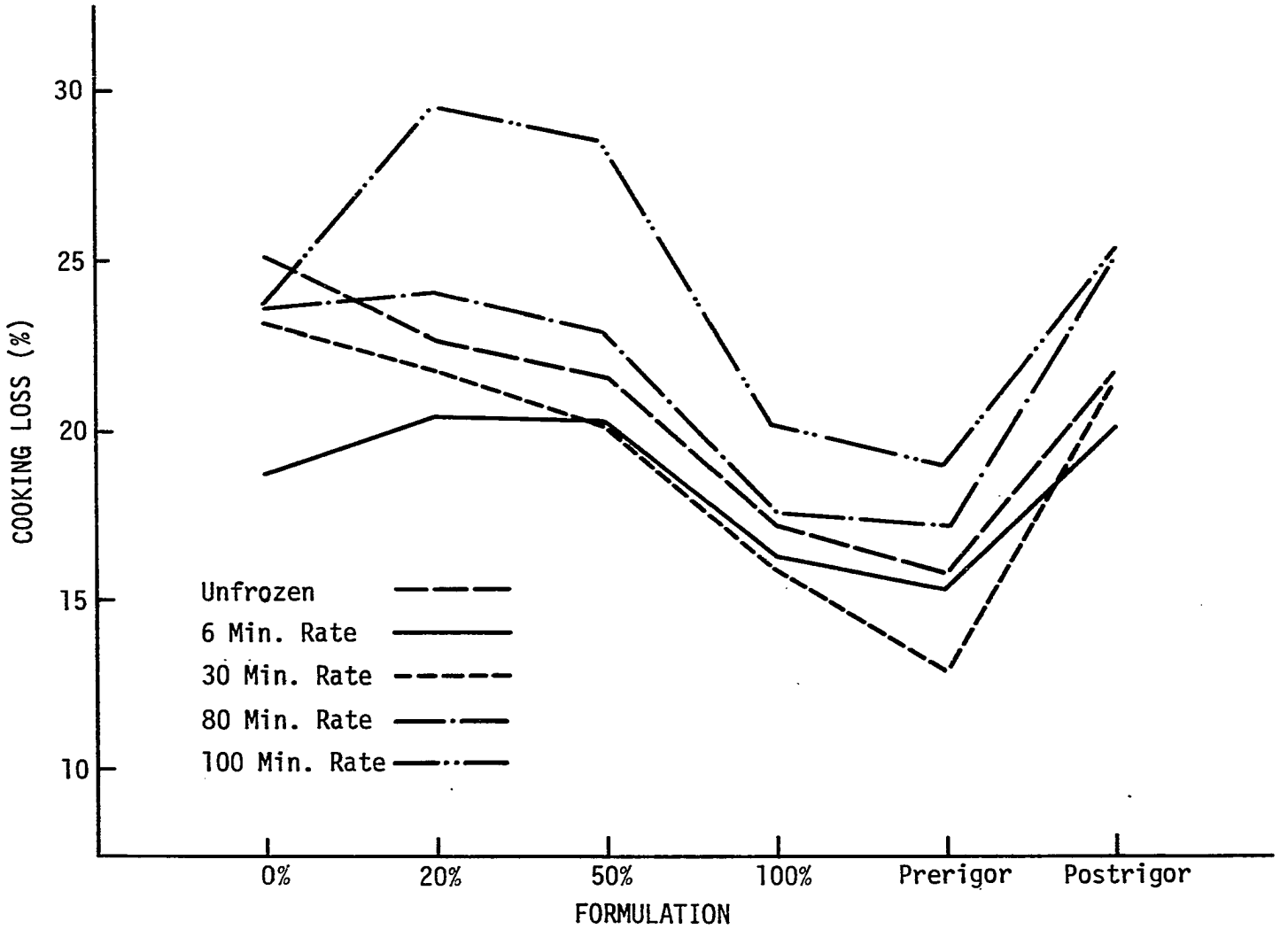

COOKING LOSS (%)
30
25
20
15
10
Unfrozen
6 Min. Rate
30 Min. Rate
80 Min. Rate
100 Min. Rate
0%
20%
50%
100%
Prerigor
Postrigor
FORMULATION

Table 11. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for percentage cooking loss[4] after thawing at 5°C

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)				Overall treatment means
	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	17.55 0.77	19.86 2.35	20.16 0.50	18.38 3.32	18.99[a] 2.20
20% Frozen trim	20.65 1.87	19.34 1.34	19.56 0.69	20.10 1.78	19.91[a] 1.47
50% Frozen trim	20.29 1.16	16.56 1.83	17.79 1.92	16.38 1.97	17.75[b] 2.27
100% Frozen trim	15.56 1.28	14.22 1.72	15.54 1.43	15.60 1.00	15.23[c] 1.40
Prerigor trim	16.90 1.41	12.28 1.79	12.35 0.58	12.13 1.53	13.42[d] 2.43
Postrigor trim	20.12 1.43	18.71 1.70	18.61 1.89	21.33 0.79	19.69[a] 1.80
Overall treatment means	18.51[a] 2.33	16.83[b] 3.28	17.33[b] 2.97	17.32[b] 3.56	

[1] n=5 observations per cell.

[2] Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3] Standard deviations are listed below each mean.

[4] Calculated weight loss as a percentage of thawed pattie weight.

[5] Frozen trim denotes frozen-flaked trim.

Table 12. Individual cell means[1], [3]main effect treatment means[2] and standard deviations[3] for percentage cooking loss[4] after thawing at 20°C

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)				Overall treatment means
	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	19.67 0.78	16.79 1.89	19.52 1.52	20.98 1.38	19.24[b] 2.05
20% Frozen trim	22.95 1.28	16.24 0.98	19.04 4.15	22.02 2.03	20.06[b] 3.51
50% Frozen trim	21.95 1.32	21.04 0.57	20.68 2.63	22.46 1.45	21.53[a] 1.69
100% Frozen trim	16.94 1.10	16.13 0.65	15.28 1.15	16.54 2.71	16.22[c] 1.60
Prerigor trim	17.67 2.22	12.98 1.02	14.01 0.80	15.43 1.19	15.02[d] 2.22
Postrigor trim	21.54 1.75	21.12 1.01	21.15 1.00	20.90 1.71	21.18[a] 1.32
Overall treatment means	20.12[a] 2.64	17.38[c] 3.10	18.28[b] 3.40	19.72[a] 3.22	

[1]n=5 observations per cell.

[2]Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3]Standard deviations are listed below each mean.

[4]Calculated weight loss as a percentage of thawed pattie weight.

[5]Frozen trim denotes frozen-flaked trim.

There was a significant ($p<0.05$) decrease in cooking loss for patties containing 100% frozen-flaked or 100% prerigor trim. This was observed at both thaw temperatures (Tables 11 and 12) and the results are similar to the patties cooked in the frozen state. A comparison of cook loss by freezing rate, over all formulations, shows no significant trends and indicate little, if any, advantage for faster freezing rates at either thaw temperature.

In an attempt to obtain further information on methods for cooking ground beef patties, overall group means for each of the four cooking treatments employed in this study were compared in Table 13. It was previously mentioned that patties frozen at 6 or 30 minute rates experienced less cook loss than unfrozen patties (Table 10). When all four freezing rates are averaged and compared to unfrozen patties (Table 13), no significant differences in cook loss were apparent. Both unfrozen patties and those cooked from the frozen state had significantly greater weight loss during cooking than either of the groups thawed to 5°C prior to grilling. This indicates an advantage for thawing patties before cooking, although this is not a common practice among major hamburger restaurants. Furthermore, a comparison of the two thaw groups shows significantly less cook loss from the patties thawed at 5°C. This would suggest that when thawing is necessary before cooking, a less rapid thaw at a cooler

Table 13. The effect of formulation[1,2] on percentage cooking loss[3] from patties cooked nonfrozen, frozen and after thawing

Cooking variable	Formulation[4]						Overall treatment means
	0% Frozen trim	20% Frozen trim	50% Frozen trim	100% Frozen trim	Pre-rigor trim	Post-rigor trim	
Cooked from unfrozen state (n=5 obs/cell)	25.24 0.21	22.69 0.57	21.73 0.74	17.29 1.11	15.90 1.92	21.64 2.02	20.75[a] 3.45
Cooked from frozen state (n=20 obs/cell)	22.29 2.87	24.00 3.74	23.01 3.73	17.48 2.62	16.10 2.83	23.00 3.18	20.98[a] 4.36
Cooked after thawing at 5°C (n=20 obs/cell)	18.99 2.20	19.91 1.47	17.75 2.27	15.23 1.40	13.42 2.43	19.69 1.80	17.50[c] 3.09
Cooked after thawing at 20°C (n=20 obs/cell)	19.24 2.05	20.06 3.51	21.53 1.69	16.22 1.60	15.02 2.22	21.17 1.32	18.88[b] 3.26
Overall treatment means	21.44[a] 3.02	21.67[a] 3.46	21.01[a] 3.35	16.56[b] 2.08	15.11[c] 2.66	21.38[a] 2.54	

[1]Standard deviations are listed below each mean.

[2]Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3]Calculated weight loss as a percentage of precooked pattie weight.

[4]Frozen trim denotes frozen-flaked trim.

temperature is desirable. A comparison of formulations in Table 13 again shows significant advantages for the 100% frozen-flaked trim and prerigor patties regardless of cooking treatment employed.

Effects of Freezing Rate and Formulation on Sensory Characteristics

Formulation comparisons

After cooking, patties were allowed to cool to room temperature before 2.54 cm core samples were removed and sheared by the Warner-Bratzler shearing device. These objective measurements of texture are given in Table 14. Subjective taste panel evaluations of texture, as well as flavor, juiciness and overall acceptability are shown in Tables 15-18 respectively.

The Warner-Bratzler values (Table 14) clearly indicate a significant decrease in resistance to shear force with greater percentages of frozen-flaked trim. The influence of flaking is further illustrated in Figure 5. Taste panel members were also able to detect greater tenderness (Table 15) in patties containing 50 and 100% frozen-flaked trim than those at the zero or 20% frozen trim levels. Patties containing 20 or 50% frozen-flaked trim were comparable in flavor (Table 16), juiciness (Table 17) and overall acceptability (Table 18) to formulations with no frozen-flaked trim.

Table 14. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for Warner-Bratzler shear values (kgs)[4]

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)					Overall treatment means
	Un-frozen	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	1.16 0.20	1.59 0.50	1.65 0.46	1.58 0.30	1.75 0.20	1.55^{a} 0.40
20% Frozen trim	1.23 0.45	1.27 0.41	1.54 0.48	1.62 0.31	1.50 0.41	1.43^{b} 0.43
50% Frozen trim	1.17 0.32	0.98 0.25	0.98 0.21	1.04 0.25	1.20 0.25	1.07^{c} 0.27
100% Frozen trim	0.61 0.21	0.45 0.11	0.46 0.10	0.65 0.20	0.70 0.13	0.58^{d} 0.18
Prerigor trim	1.48 0.42	1.06 0.31	1.40 0.35	1.40 0.42	2.05 0.38	$1.48^{a,b}$ 0.49
Postrigor trim	1.18 0.27	1.29 0.44	1.43 0.24	1.51 0.27	1.79 0.24	$1.44^{a,b}$ 0.36
Overall treatment means	1.14^{c} 0.41	1.11^{c} 0.50	1.25^{b} 0.52	1.30^{b} 0.45	1.36^{a} 0.52	

[1] n=15 observations per cell.
[2] Treatment means in a column or row with the same superscripts are not significatnly different ($p<0.05$).
[3] Standard deviations are listed below each mean.
[4] Smaller values indicate less resistance to the shear force.
[5] Frozen trim denotes frozen-flaked trim.

Figure 5. Effect of pattie formulation and freezing rate on shear values. Formulation percentages indicate amount of frozen-flaked trim. Unfrozen line refers to all patties not frozen after formulation

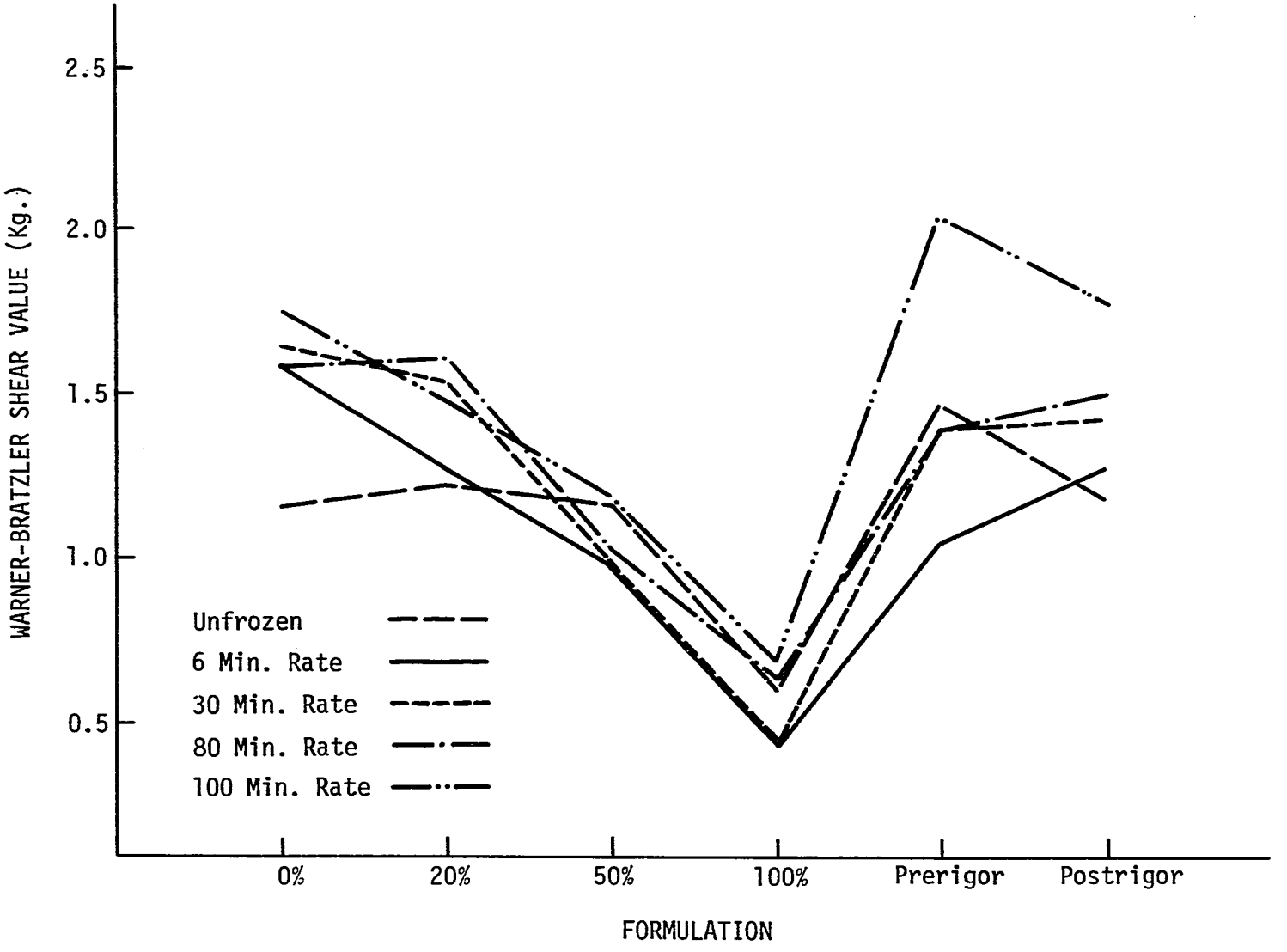

WARNER-BRATZLER SHEAR VALUE (Kg.)
2.5
2.0
1.5
1.0
0.5
Unfrozen
6 Min. Rate
30 Min. Rate
80 Min. Rate
100 Min. Rate
0%
20%
50%
100%
Prerigor
Postrigor
FORMULATION

Table 15. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for sensory evaluation of texture[4]

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)					Overall treatment means
	Unfrozen	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	6.06 1.47	6.56 1.50	6.11 1.94	6.00 1.37	5.00 1.58	$6.00^{b,c}$ 1.62
20% Frozen trim	7.00 1.28	6.92 1.16	6.50 1.31	6.00 1.76	4.92 1.93	$6.23^{a,b}$ 1.66
50% Frozen trim	7.38 0.77	6.85 1.14	7.23 1.42	6.08 1.38	5.31 1.89	6.57^{a} 1.54
100% Frozen trim	5.86 1.46	6.88 1.32	6.41 1.37	6.65 1.41	6.65 1.32	6.51^{a} 1.38
Prerigor trim	5.33 1.37	6.17 1.34	5.58 1.73	5.58 1.68	5.33 1.50	5.60^{c} 1.51
Postrigor trim	6.46 1.51	7.08 1.04	6.92 1.19	5.69 1.18	5.62 1.19	$6.35^{a,b}$ 1.34
Overall treatment means	$6.33^{a,b}$ 1.47	6.74^{a} 1.27	$6.45^{a,b}$ 1.58	6.04^{b} 1.46	5.54^{c} 1.65	

[1] n=12 to 18 observations per cell.

[2] Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3] Standard deviations are listed below each mean.

[4] Based on hedonic scale: 9 = extremely tender; 1 = extremely tough.

[5] Frozen trim denotes frozen-flaked trim.

Table 16. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for sensory evaluation of flavor[4]

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)					Overall treatment means
	Unfrozen	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	6.72 1.64	6.50 1.38	6.11 1.23	6.67 0.91	5.77 1.01	$6.39^{a,b}$ 1.29
20% Frozen trim	6.08 1.68	6.92 1.16	7.00 1.28	6.33 1.30	6.17 1.34	6.50^{a} 1.37
50% Frozen trim	5.92 1.66	6.23 1.42	6.31 1.49	6.54 1.20	5.31 1.70	$6.06^{a,b,c}$ 1.52
100% Frozen trim	4.21 1.81	5.65 1.11	6.53 1.07	6.12 1.05	5.29 1.26	5.61^{c} 1.46
Prerigor trim	6.50 1.17	5.75 1.22	6.17 1.27	6.08 1.24	6.42 1.17	$6.18^{a,b}$ 1.20
Postrigor trim	5.92 1.66	6.15 1.46	5.92 1.44	5.77 1.42	6.08 1.19	$5.97^{b,c}$ 1.40
Overall treatment means	$5.92^{a,b}$ 1.78	$6.19^{a,b}$ 1.33	6.33^{a} 1.30	6.27^{a} 1.18	5.80^{b} 1.33	

[1] n=12 to 18 observations per cell.
[2] Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).
[3] Standard deviations are listed below each mean.
[4] Based on hedonic scale: 9 = extremely desirable; 1 = extremely undesirable.
[5] Frozen trim denotes frozen-flaked trim.

Table 17. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for sensory evaluation of juiciness[4]

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)					Overall treatment means
	Unfrozen	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	5.72 1.57	6.61 1.33	6.00 2.00	6.17 1.25	5.46 1.56	$6.02^{c,d}$ 1.57
20% Frozen trim	7.83 0.84	7.08 0.67	6.75 1.36	6.67 1.30	5.67 1.37	6.80^{a} 1.31
50% Frozen trim	7.77 0.83	6.77 0.83	7.15 1.41	6.15 1.82	5.08 2.14	$6.58^{a,b}$ 1.72
100% Frozen trim	6.00 1.71	5.82 1.51	5.24 1.35	6.24 1.56	5.12 1.22	5.67^{d} 1.50
Prerigor trim	6.08 1.38	6.08 1.31	5.67 1.83	5.50 1.62	5.67 1.15	$5.80^{c,d}$ 1.45
Postrigor trim	6.08 1.75	7.54 0.97	6.69 0.95	5.85 1.68	5.00 1.15	$6.23^{b,c}$ 1.56
Overall treatment means	$6.51^{a,b}$ 1.63	6.61^{a} 1.28	$6.19^{a,b}$ 1.64	6.11^{b} 1.53	5.31^{c} 1.45	

[1] n=12 to 18 observations per cell.

[2] Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).

[3] Standard deviations are listed below each mean.

[4] Based on hedonic scale: 9 = extremely juicy; 1 = extremely dry.

[5] Frozen trim denotes frozen-flaked trim.

Table 18. Individual cell means[1], main effect treatment means[2] and standard deviations[3] for sensory evaluation of overall acceptability[4]

Formulation[5]	Freezing rates (Time for +5°C to -5°C change)					Overall treatment means
	Unfrozen	6 min.	30 min.	80 min.	100 min.	
0% Frozen trim	6.06 1.51	6.33 1.41	5.78 1.56	6.33 0.84	5.62 1.12	6.05[a] 1.33
20% Frozen trim	6.67 1.56	6.83 1.11	6.75 1.22	6.17 1.27	5.50 1.38	6.38[a] 1.37
50% Frozen trim	6.54 1.71	6.23 1.54	6.54 1.66	6.23 1.17	5.23 1.74	6.15[a] 1.60
100% Frozen trim	4.50 1.87	5.53 1.18	6.00 1.06	6.12 1.27	5.41 1.37	5.55[b] 1.43
Prerigor trim	5.92 1.00	5.92 1.08	6.00 1.60	5.67 1.15	5.75 1.48	5.85[a,b] 1.25
Postrigor trim	6.08 1.55	6.62 1.33	6.00 1.47	5.62 1.26	5.38 1.19	5.94[a,b] 1.39
Overall treatment means	5.94[a] 1.67	6.21[a] 1.33	6.14[a] 1.43	6.05[a] 1.15	5.48[b] 1.36	

[1]n=12 to 18 observations per cell.
[2]Treatment means in a column or row with the same superscripts are not significantly different ($p<0.05$).
[3]Standard deviations are listed below each mean.
[4]Based on hedonic scale: 9 = like extremely; 1 = dislike extremely.
[5]Frozen trim denotes frozen-flaked trim.

Therefore, it appears that the addition of up to 50% frozen-flaked trim in the pattie formulation will not reduce quality from that associated with all fresh, nonfrozen trim.

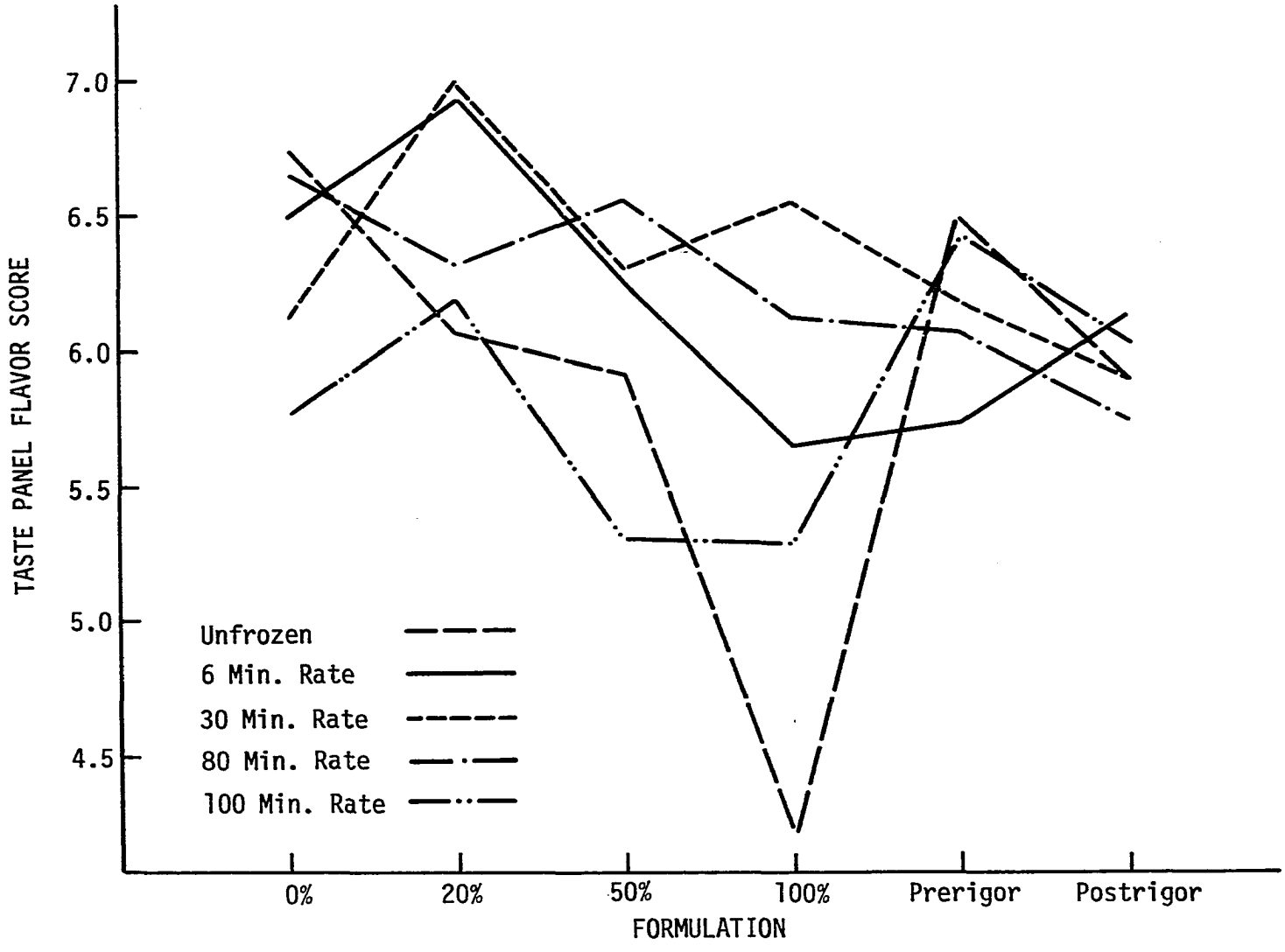
However, patties formulated from 100% frozen-flaked trim were ranked lower for flavor (Table 16, Figure 6) and overall acceptability (Table 18) than formulations with 0% frozen-flaked trim. Although juiciness values (Table 17) were lower for the 100% frozen-flaked pattie, they were not significantly different from the 0% frozen-flaked formulation. These data suggest that while flaked levels of up to 50% may be acceptable to consumers, increased levels beyond this point may sacrafice some quality and should be used with caution.

Except for taste panel texture values (Table 15), prerigor patties were not significantly different from postrigor patties for any of the other sensory traits evaluated. This would indicate that prerigor meat, when processed under proper conditions, can be utilized successfully in the production of frozen ground beef patties.

Freezing rate comparisons

Warner-Bratzler shear values shown in Table 14 clearly indicate that faster freezing rates increase pattie tenderness. This supports results reported by numerous investigators (Hankins and Hiner, 1940; Hiner and Hankins, 1946; Guenther

Figure 6. Effect of pattie formulation and freezing rate on taste panel flavor scores. Formulation percentages indicate amount of frozen-flaked trim. Unfrozen line refers to all patties not frozen after formulation


TASTE PANEL FLAVOR SCORE
7.0
6.5
6.0
5.5
5.0
4.5
Unfrozen
6 Min. Rate
30 Min. Rate
80 Min. Rate
100 Min. Rate
0%
20%
50%
100%
Prerigor
Postrigor
FORMULATION

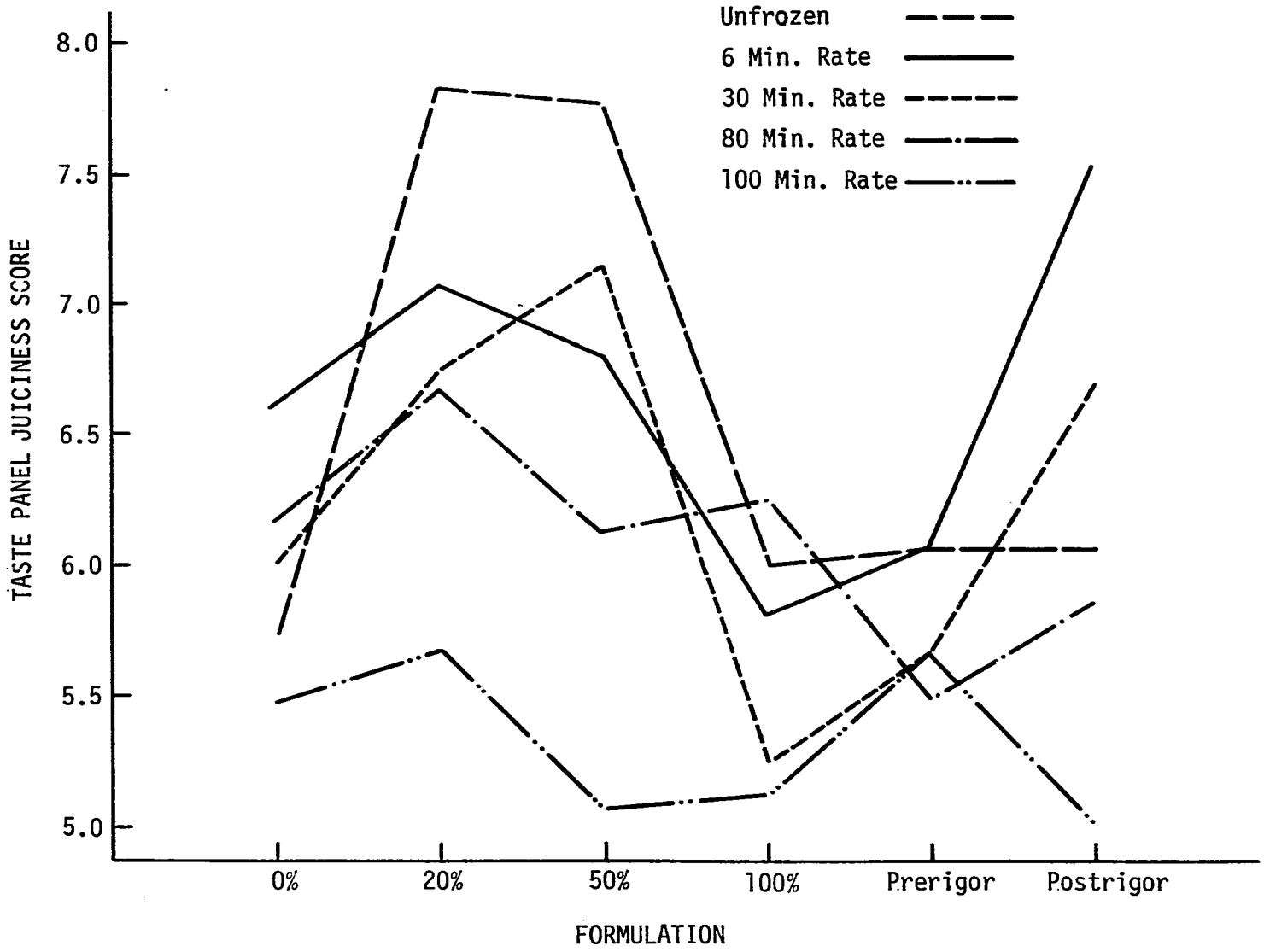
and Henrickson, 1962; Sebranek et al., 1978). As additional evidence, the taste panel score for texture (5.54, Table 15) was significantly lower than those scores at the three faster freezing rates (6.04, 6.45 and 6.74). Neither the Warner-Bratzler shear method nor taste panel evaluation could detect texture differences between unfrozen patties and those frozen at the 6 minute rate. This suggests that freezing at this rapid rate does not adversely affect product tenderness.

Taste panel results indicated essentially no significant differences in flavor of the patties at the different freezing rates (Table 16). Similar results were reported by Footrakul (1976).

Juiciness scores (Table 17) were significantly ($p<0.05$) lower for samples frozen at the 100 minute rate. The consistently lower values for juiciness at the slowest freezing rate are observed in Figure 7. Ryan (1966), Gray (1967) and Sebranek et al. (1978) have also reported higher juiciness scores for products frozen at faster rates of freezing. Juiciness is also significantly ($p<0.01$) and negatively correlated ($r=-0.26$) to shrink loss (Table 19). Evidently, greater moisture loss during freezing, combined with the subsequent moisture loss during cooking, reduces pattie juiciness as measured by the taste panel.

Overall acceptability scores (Table 18) are an expression by the taste panel of total pattie quality. Values recorded

Figure 7. Effect of pattie formulation and freezing rate on taste panel juiciness scores. Formulation percentages indicate amount of frozen-flaked trim. Unfrozen line refers to all patties not frozen after formulation


Unfrozen
6 Min. Rate
30 Min. Rate
80 Min. Rate
100 Min. Rate
TASTE PANEL JUICINESS SCORE
8.0
7.5
7.0
6.5
6.0
5.5
5.0
0%
20%
50%
100%
Prerigor
Postrigor
FORMULATION

by this taste panel indicate that patties frozen at 6, 30 or 80 minute rates are more acceptable than patties frozen at the 100 minute, or slowest freezing rate. It is apparent that there is a point at which slower freezing rates significantly reduce overall pattie acceptability. That point is between the 80 and 100 minute freezing rate.

Simple correlations among all variables studied are shown in Table 19. Mean squares of the analysis of variance for all traits discussed are listed in Appendix A (Tables 21-29).

Effects of Freezing Rate and Formulation on Microstructure (LM and SEM) of Unfrozen, Frozen and Cooked Patties

All of the pattie samples evaluated microscopically in this study were prepared without chemical fixation techniques. Once frozen, patties remained in that state for freeze-drying or freeze-microtoming procedures. Every attempt was made to prevent recrystallization during sample removal and preparation to ensure that cavities seen in the freeze-dried (SEM) or freeze-microtomed (LM) samples were replicas of the ice crystals formed in the initial freezing. Techniques used for sample preparation in this study have been utilized successfully by several other workers investigating structural damage caused by freezing (Koonz and Ramsbottom, 1939; Jensen, 1954; Wang et al., 1954; Menz and Luyet, 1961; Boyd, 1978).

Unfrozen formulation comparisons (LM and SEM)

LM and SEM micrographs of the six unfrozen pattie formulations are shown in Figures 8-10. LM micrographs of the four frozen-flaked/nonfrozen trim formulations are shown in Figure 8 with their corresponding SEM micrographs shown in Figure 9. The comminution effect of flaking is clearly apparent when comparing 0% frozen-flaked formulations (Figures 8 and 9,A) with 100% frozen-flaked patties (Figures 8 and 9,D). Intact muscle fibers (30-70 μm diam.), nuclei and distinct fat globules are readily visible at the zero and 20% frozen-flaked levels. As the percentage of frozen-flaked trim increases, muscle fibers and fat globules lose individual integrity and become homogeneous in nature (Figures 8 and 9,C and D). Although flaking increased tenderness (Table 14) and WHC (Table 3), and decreased cook loss (Table 10), patties with greater than 50% frozen-flaked trim were less acceptable to the taste panel (Table 18) and were much less cohesive during grilling. This may be associated with the extreme structural differences observed in the 100% frozen-flaked patties.

No significant structural differences were observed between prerigor and postrigor formulations with either LM or SEM (Figure 10, A-D). Also, since 0% frozen-flaked and postrigor formulations were essentially the same, as shown

Figure 8. Light micrographs of unfrozen ground beef patties; stained with oil red 0 and hematoxylin. X 120

A) Formulation: 0% frozen-flaked trim

B) Formulation: 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation: 50% frozen-flaked trim, 50% nonfrozen trim

D) Formulation: 100% frozen-flaked trim

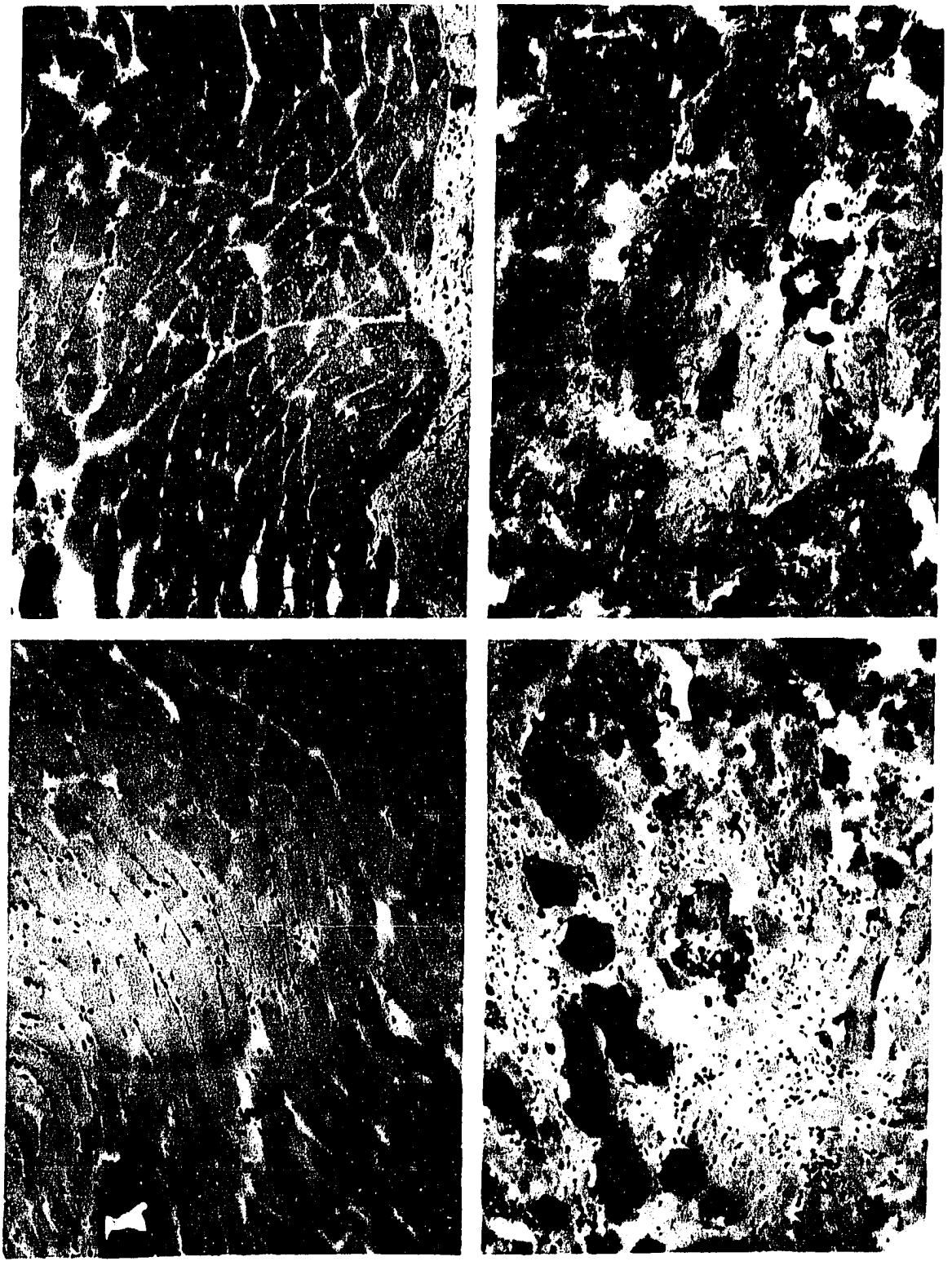


Figure 9. SEM micrographs of unfrozen ground beef patties; freeze-dried. X 120

A) Formulation: 0% frozen-flaked trim

B) Formulation: 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation: 50% frozen-flaked trim, 50% nonfrozen trim

D) Formulation: 100% frozen-flaked trim

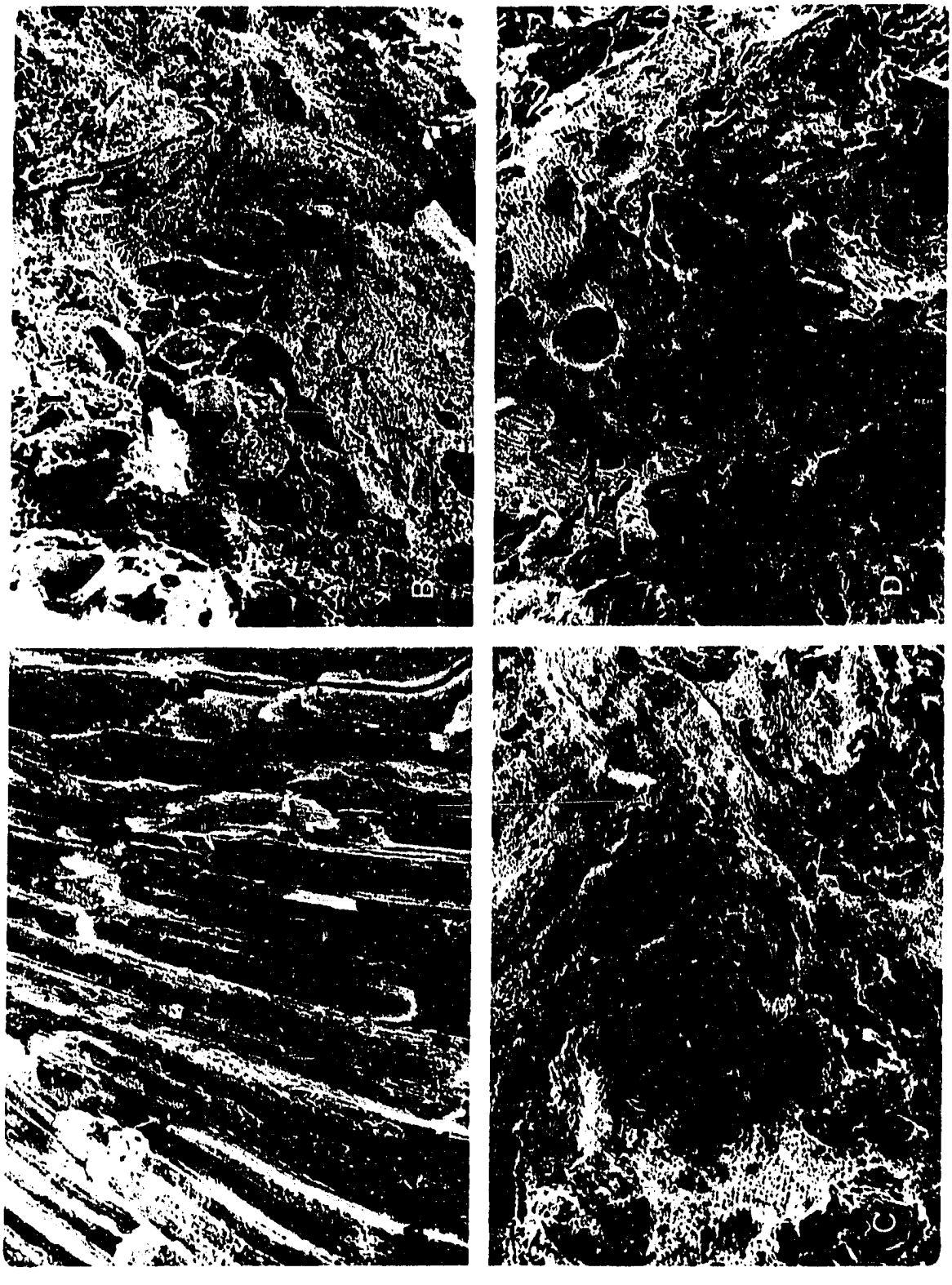

B
D
C

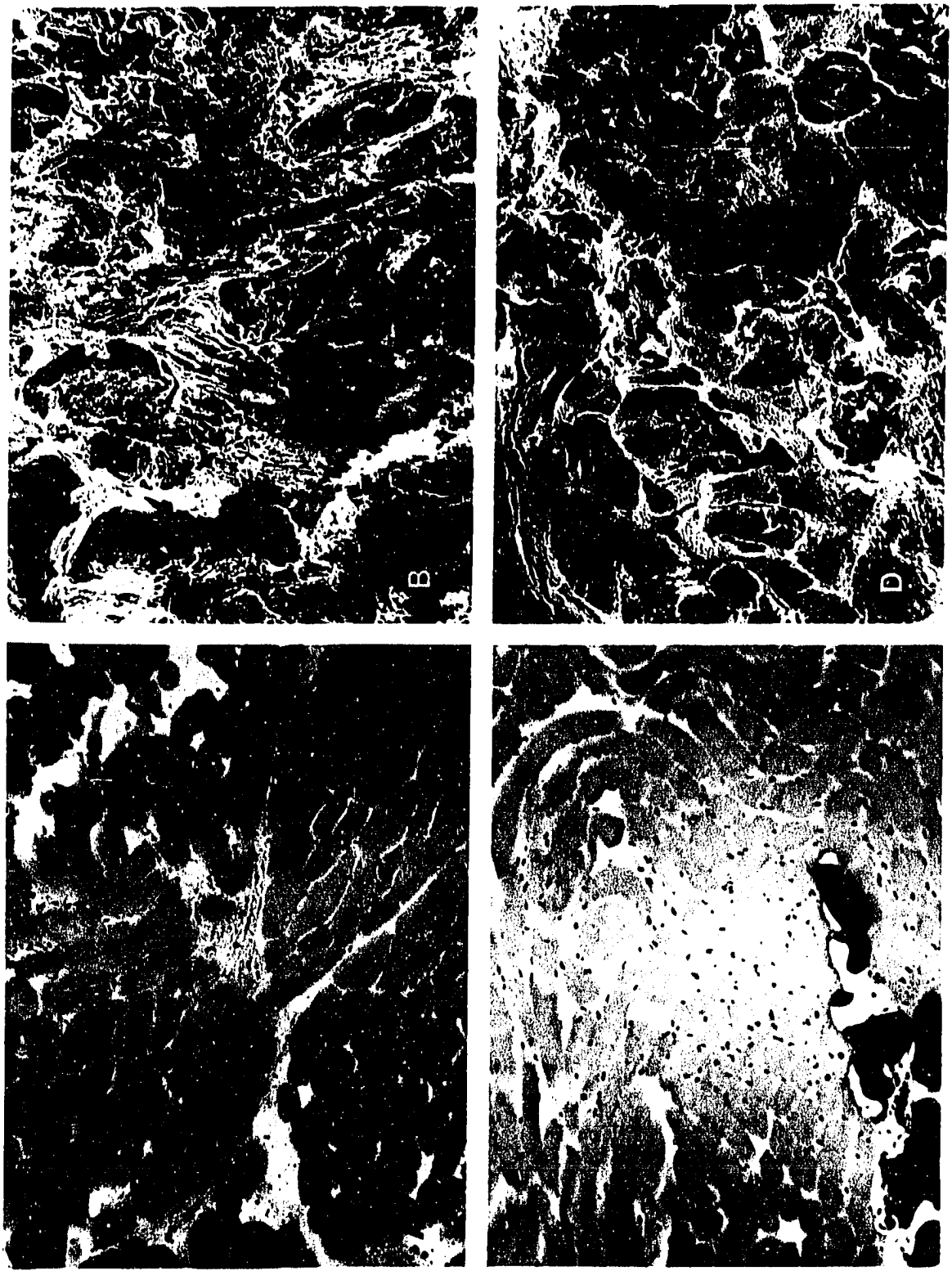
Figure 10. Micrographs (LM & SEM) of unfrozen ground beef patties. X 120

A) Formulation: 100% prerigor trim; stained with oil red 0 and hematoxylin (LM)

B) Formulation: 100% prerigor trim; freeze-dried (SEM)

C) Formulation: 100% postrigor trim; stained with oil red 0 and hematoxylin (LM)

D) Formulation: 100% postrigor trim; freeze-dried (SEM)


B
D

qualitatively and structurally, no other postrigor micrographs were used for these comparisons.

Freezing rate comparisons (LM and SEM)

LM comparisons of freezing rate effect on each pattie formulation (0, 20, 50, 100% frozen-flaked and prerigor) are shown in Figures 11-15. Corresponding SEM micrographs are shown in Figures 16-20.

The obvious increase in ice crystal cavity size from the 6 minute to the 100 minute rate is quite evident for each formulation (A-D for Figures 11-20). It is, indeed, apparent that slower rates of freezing result in much larger ice crystals which cause muscle fibers, nuclei and fat globules to be clumped together. These micrographs illustrate the suggested concepts reported by Moran (1932), Koonz and Ramsbottom (1939), Hiner et al. (1945), Meryman (1956) and Love (1966). Several examples of the unidirectional development of ice crystals (Menz and Luyet, 1961) are shown in Figures 12,B; 13,B; 15,D; 16,A; 17,A,C; and 18,A,C.

The significantly greater cook loss experienced with slower freezing rates (Table 10) may be directly attributed to the larger ice crystal size. Larger crystals, resulting from slower freezing rates, have decreased surface area in contact with pattie tissue than the numerous, smaller ice crystals formed in rapid freezing. It appears that during

Figure 11. Light micrographs of frozen ground beef patties formulated with 0% frozen-flaked trim; stained with oil red O and hematoxylin. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

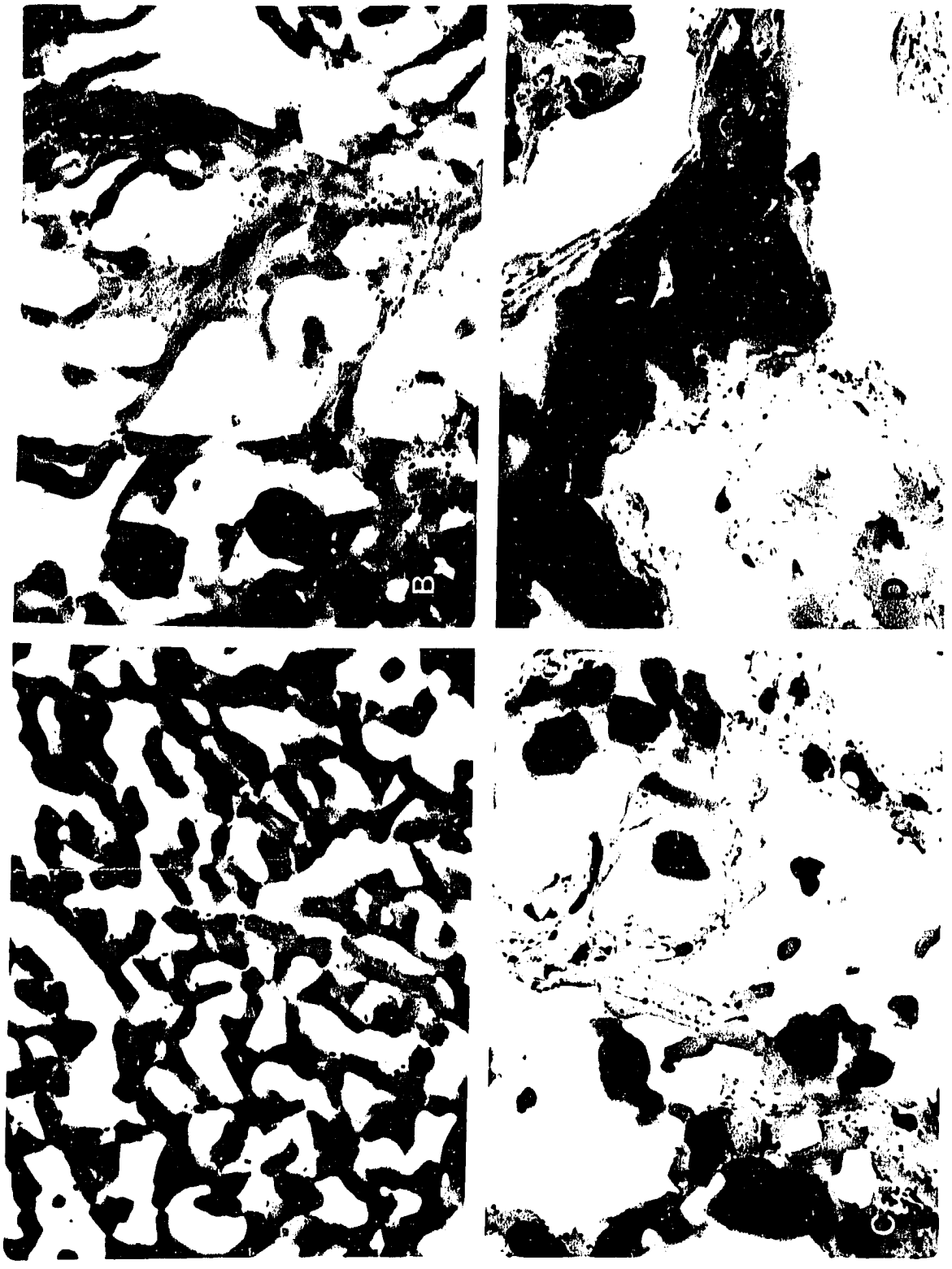

B
C

Figure 12. Light micrographs of frozen ground beef patties formulated with 20% frozen-flaked trim and 80% nonfrozen trim; stained with oil red 0 and hemotoxylin. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

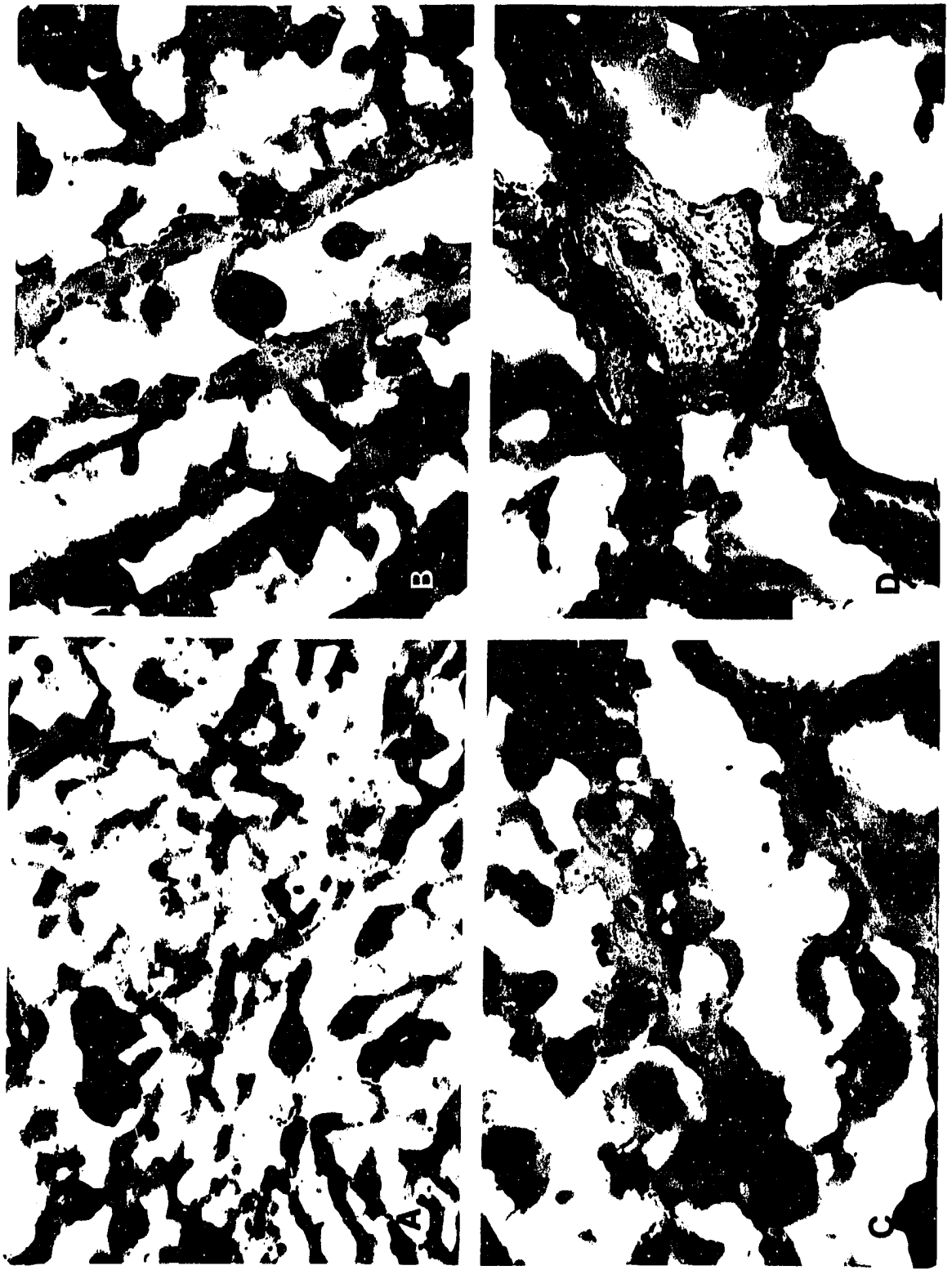

B
D
A
C

Figure 13. Light micrographs of frozen ground beef patties formulated with 50% frozen-flaked trim and 50% nonfrozen trim; stained with oil red O and hematoxylin. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

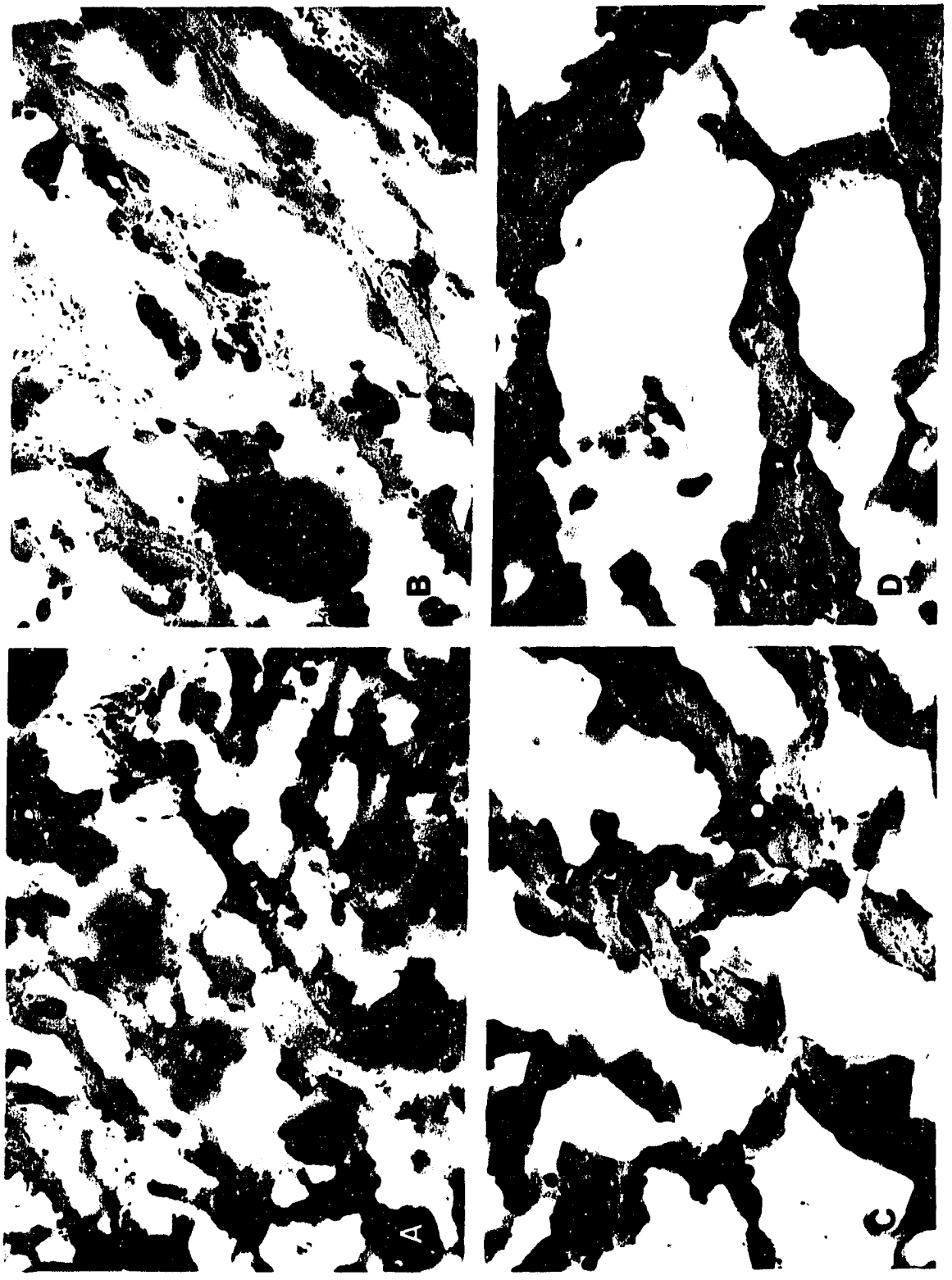

B
D
A
C

Figure 14. Light micrographs of frozen ground beef patties formulated with 100% frozen-flaked trim; stained with oil red 0 and hematoxylin. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

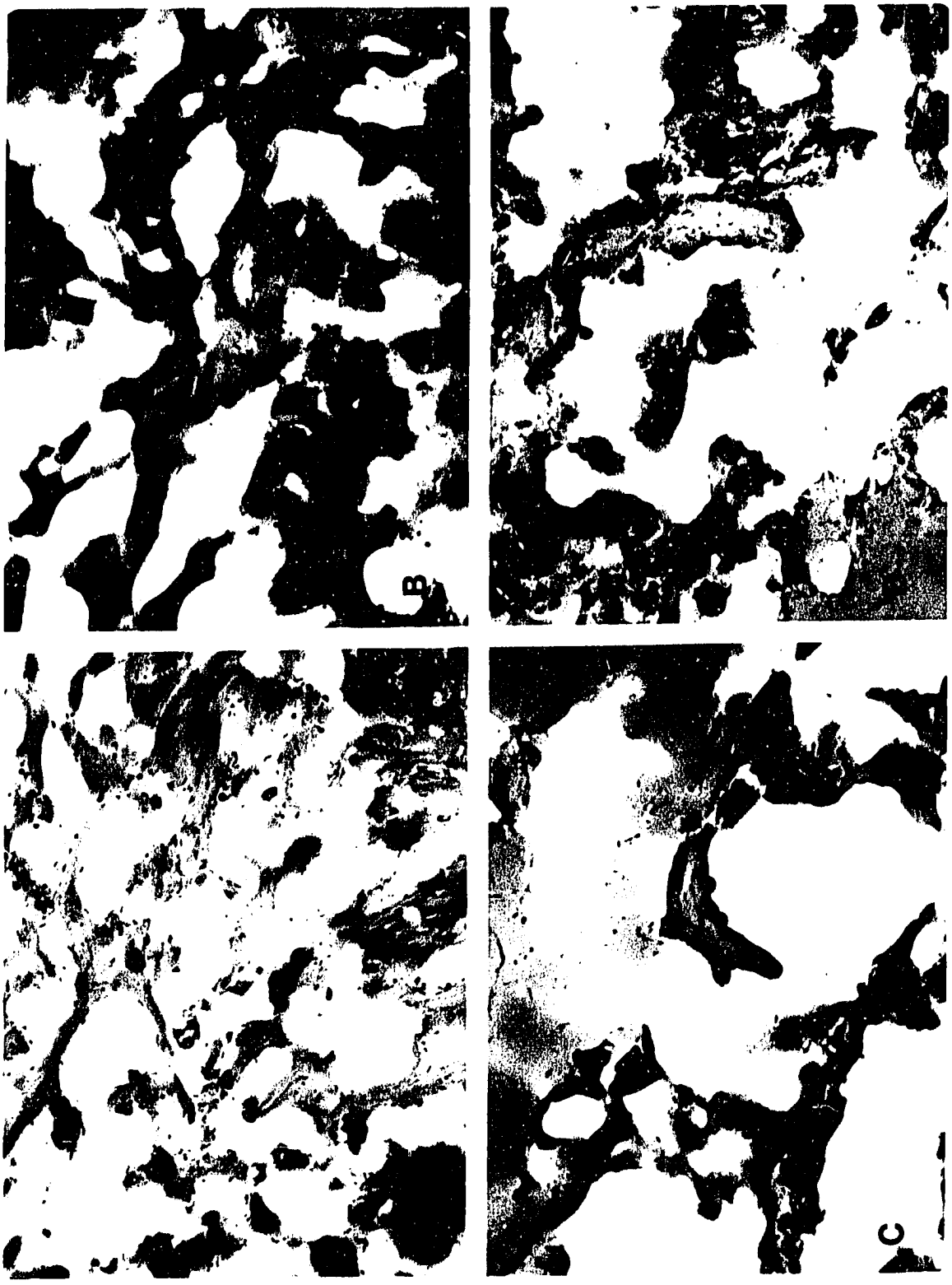

B
C

Figure 15. Light micrographs of frozen ground beef patties formulated with 100% prerigor trim; stained with oil red 0 and hematoxylin. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

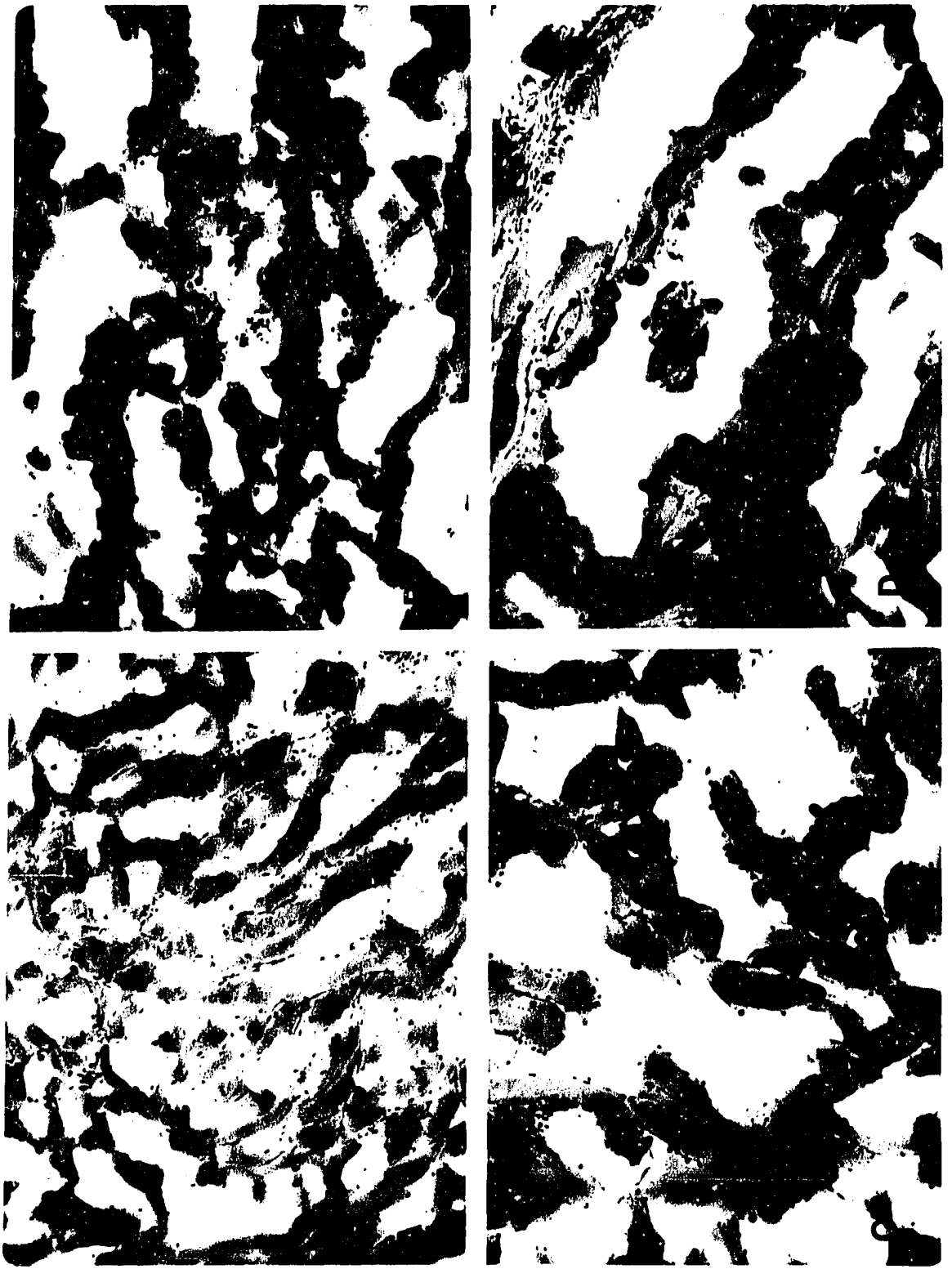


Figure 16. SEM micrographs of frozen ground beef patties formulated with 0% frozen-flaked trim; freeze-dried. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

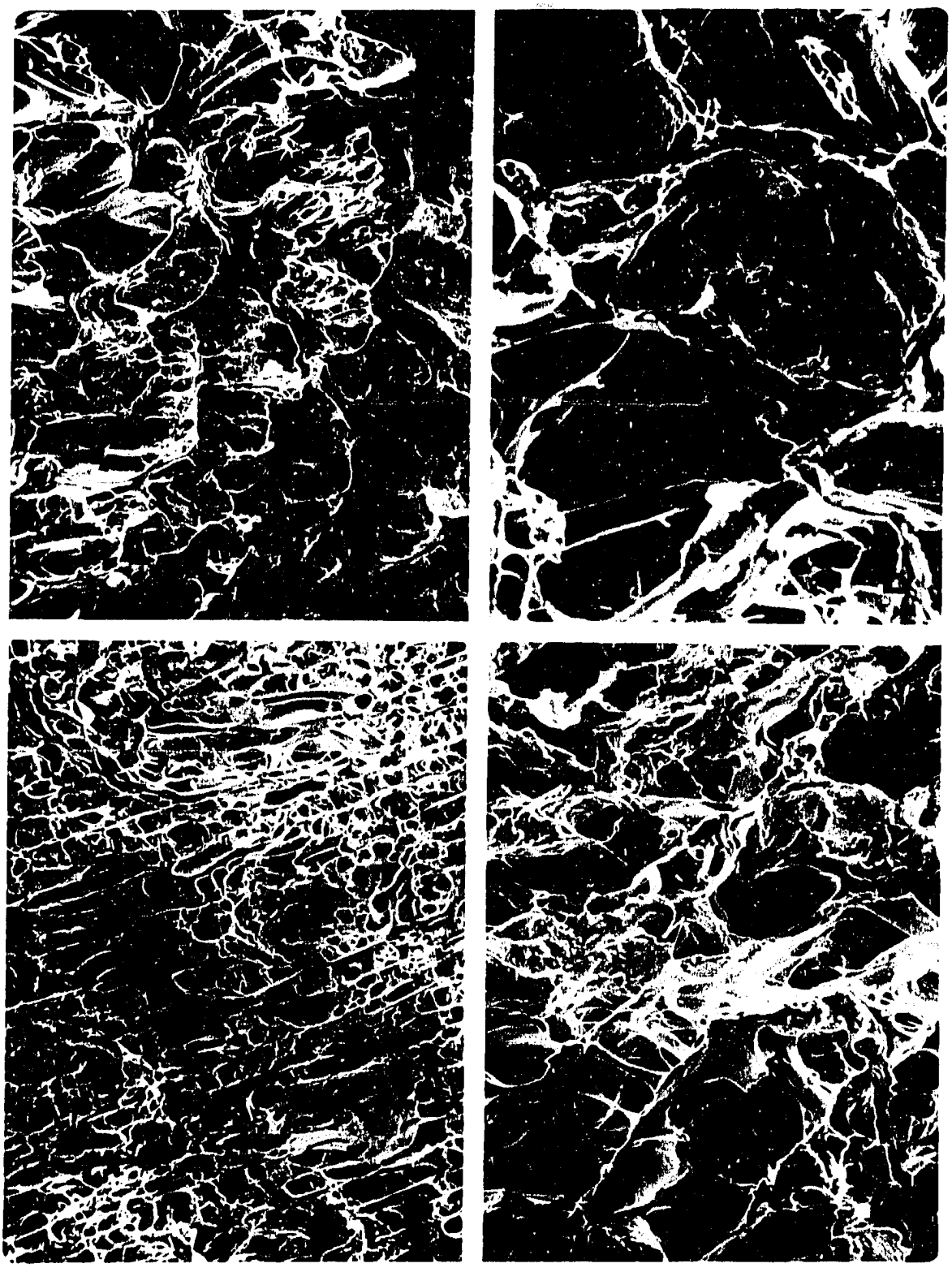


Figure 17. SEM micrographs of frozen ground beef patties formulated with 20% frozen-flaked trim and 80% nonfrozen trim; freeze-dried. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

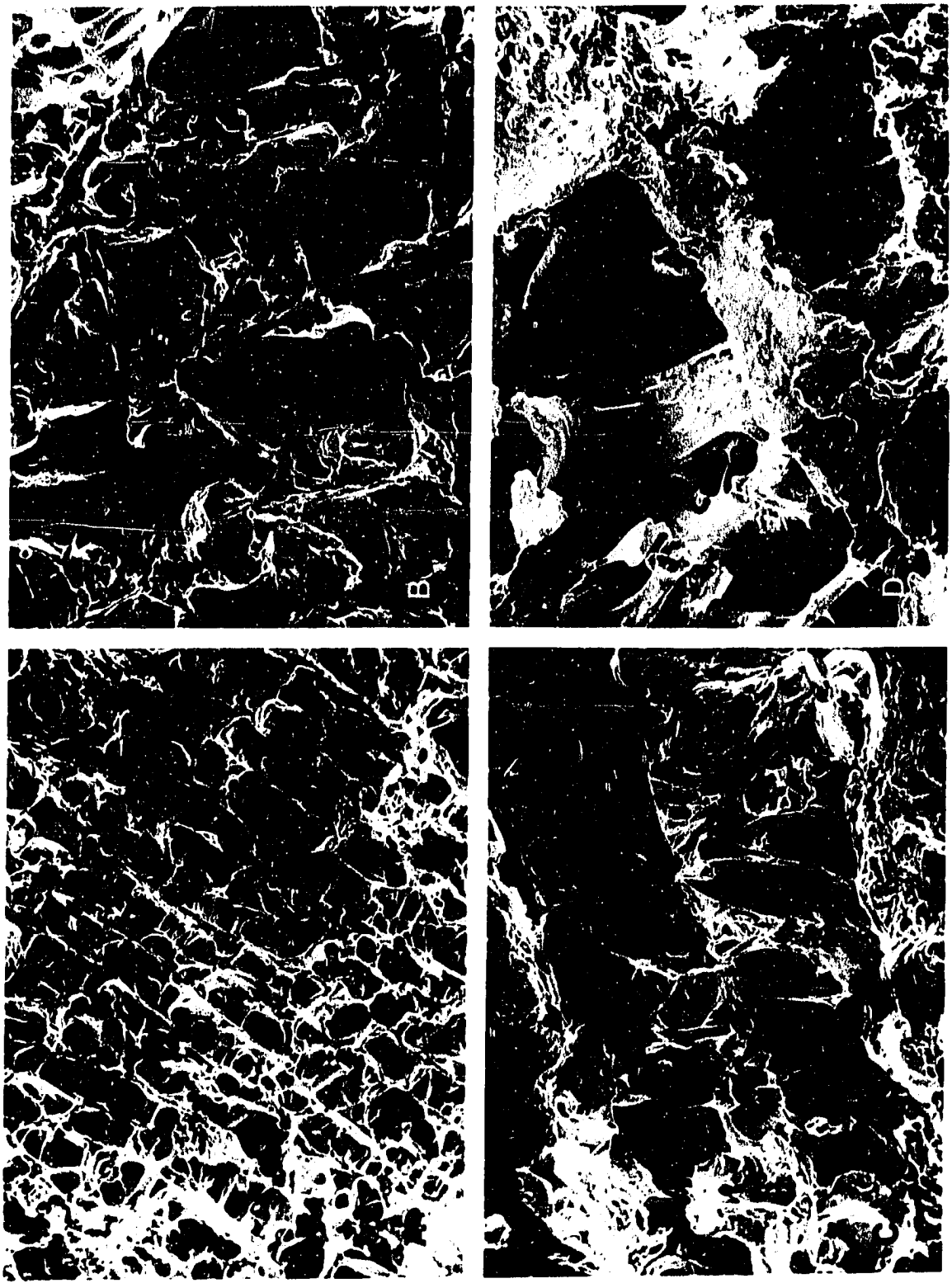

B
D

Figure 18. SEM micrographs of frozen ground beef patties formulated with 50% frozen-flaked and 50% nonfrozen trim; freeze-dried. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

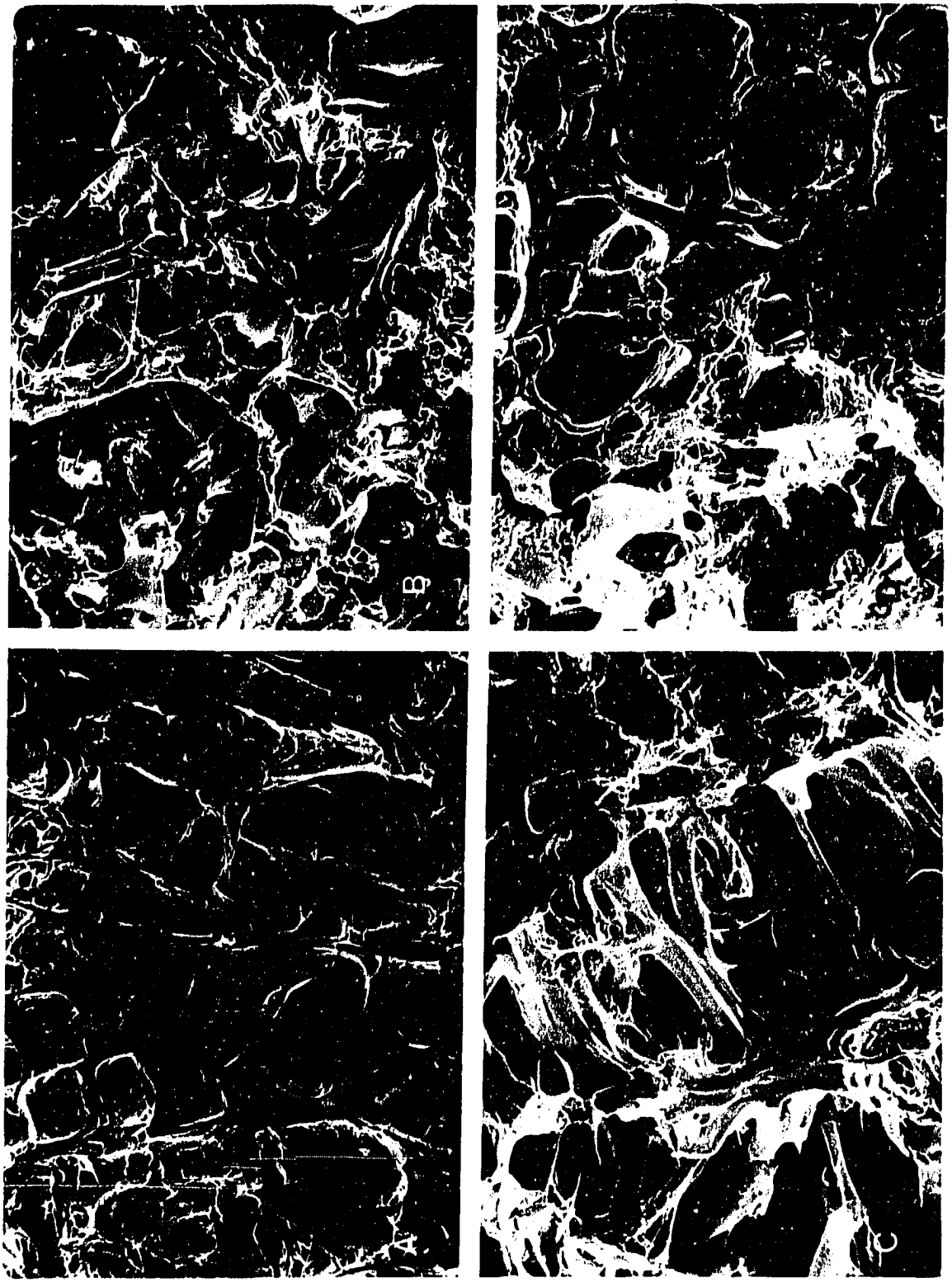

B
C

Figure 19. SEM micrographs of frozen ground beef patties formulated with 100% frozen-flaked trim; freeze dried. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

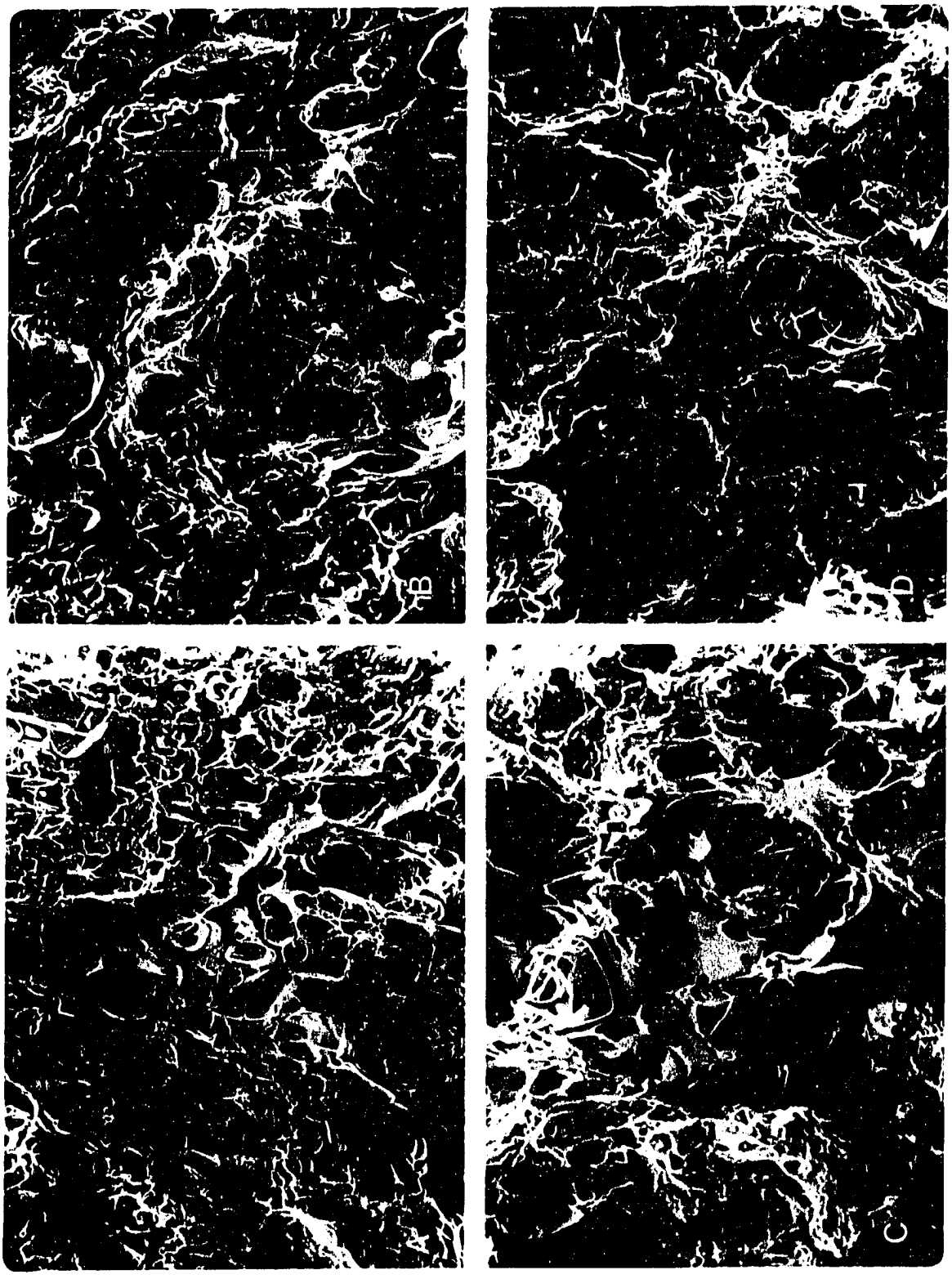

B
D
E
C

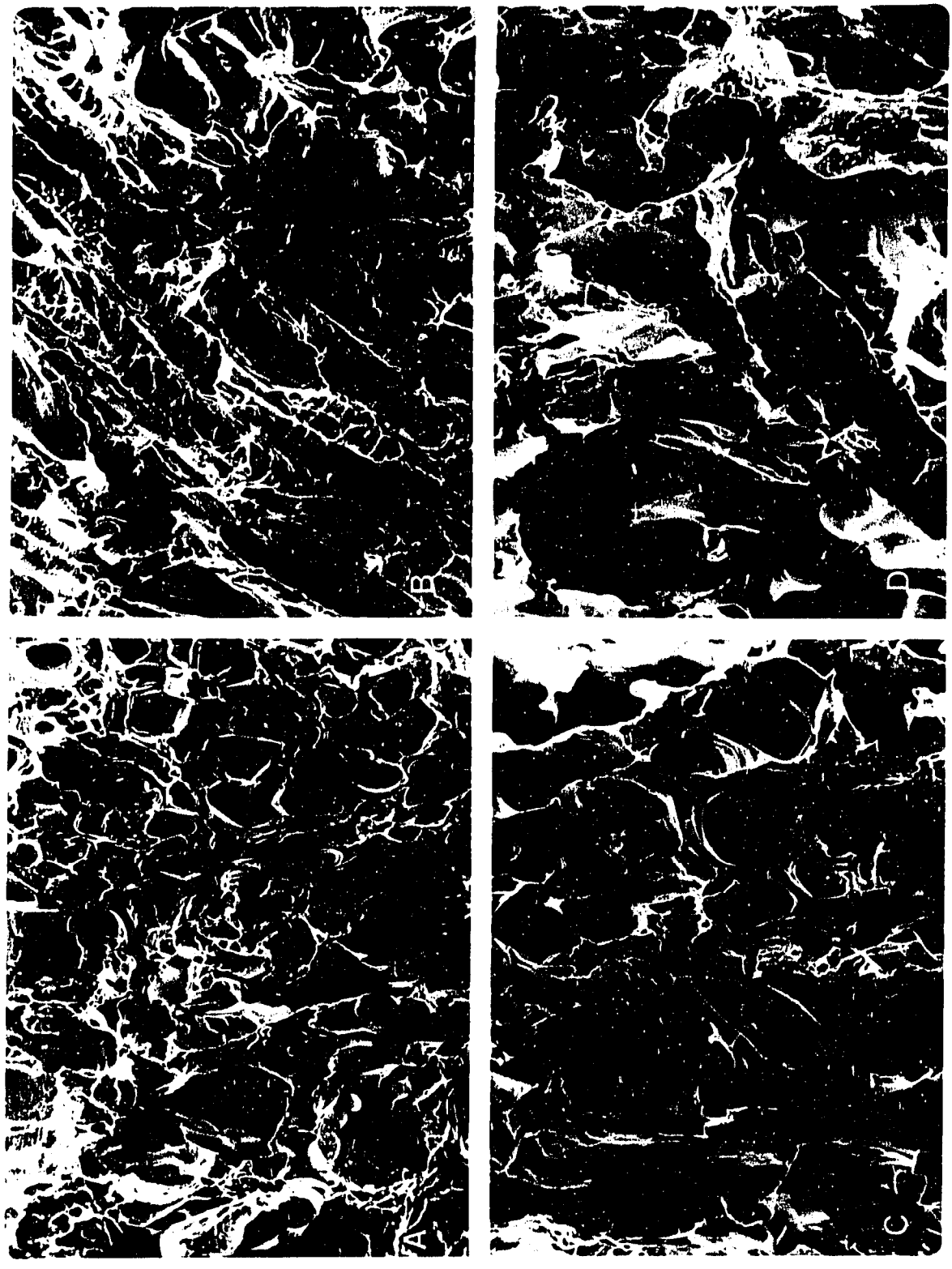
Figure 20. SEM micrographs of frozen ground beef patties formulated with 100% prerigor trim; freeze-dried. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes


B
D
A
C

rapid thawing (cooking), water produced from the larger crystals does not have time to migrate back to binding sites which were dehydrated during slow freezing. Ultimately, this water is more easily lost during cooking. This increased moisture loss at slower freezing rates could also explain the significantly lower juiciness scores (Table 17) for slower frozen patties. Also, greater moisture loss during cooking may result in increased protein denaturation which would reduce tenderness. Both objective and subjective measurements of pattie texture (Tables 14 and 15) recorded significantly lower scores at the slowest freezing rate.

Micrographs indicate that freezing rate differences became less detectable as greater amounts of frozen trim were used in the pattie formulation. This is particularly evident for the 50 and 100% frozen-flaked trim formulations (Figures 13, 14, 18 and 19). No apparent structural differences were noted between the 0% frozen-flaked trim and prerigor trim formulations.

Frozen formulation comparisons (LM and SEM)

LM micrograph comparisons of the four frozen-flaked/nonfrozen trim formulations are shown for each freezing rate in Figures 21-24. Corresponding SEM micrographs are shown in Figures 25-28. An exact interpretation of possible formulation differences within each freezing rate is difficult.

Figure 21. Light micrographs of ground beef patties frozen at a 6 minute freezing rate; stained with oil red 0 and hematoxylin. X 120

A) Formulation: 0% frozen-flaked trim

B) Formulation: 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation: 50% frozen-flaked trim, 50% nonfrozen trim

D) Formulation: 100% frozen-flaked trim

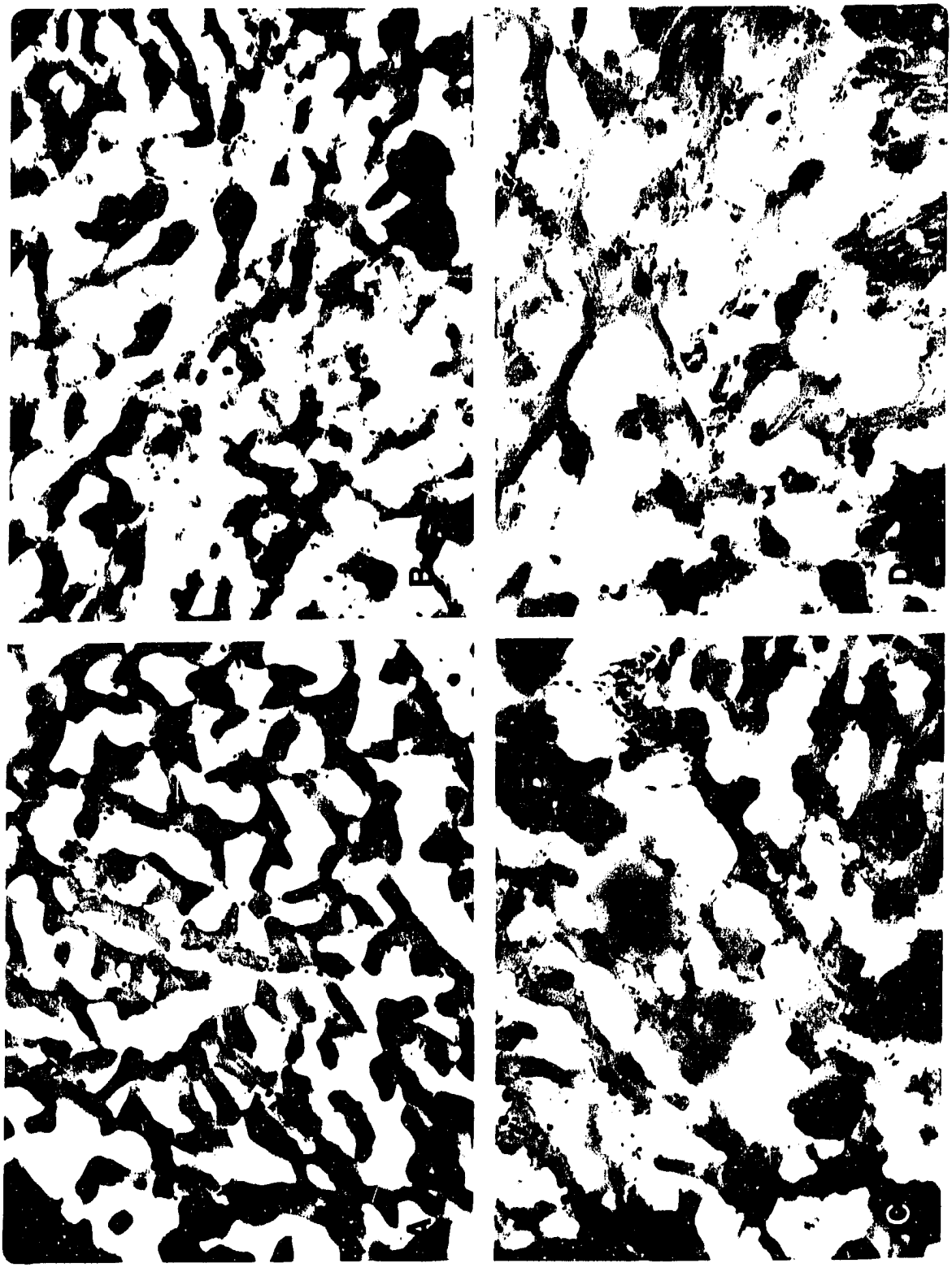

B
D
C

Figure 22. Light micrographs of ground beef patties frozen at a 30 minute freezing rate; stained with oil red 0 and hematoxylin. X 120

A) Formulation: 0% frozen-flaked trim

B) Formulation: 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation: 50% frozen-flaked trim, 50% nonfrozen trim

D) Formulation: 100% frozen-flaked trim

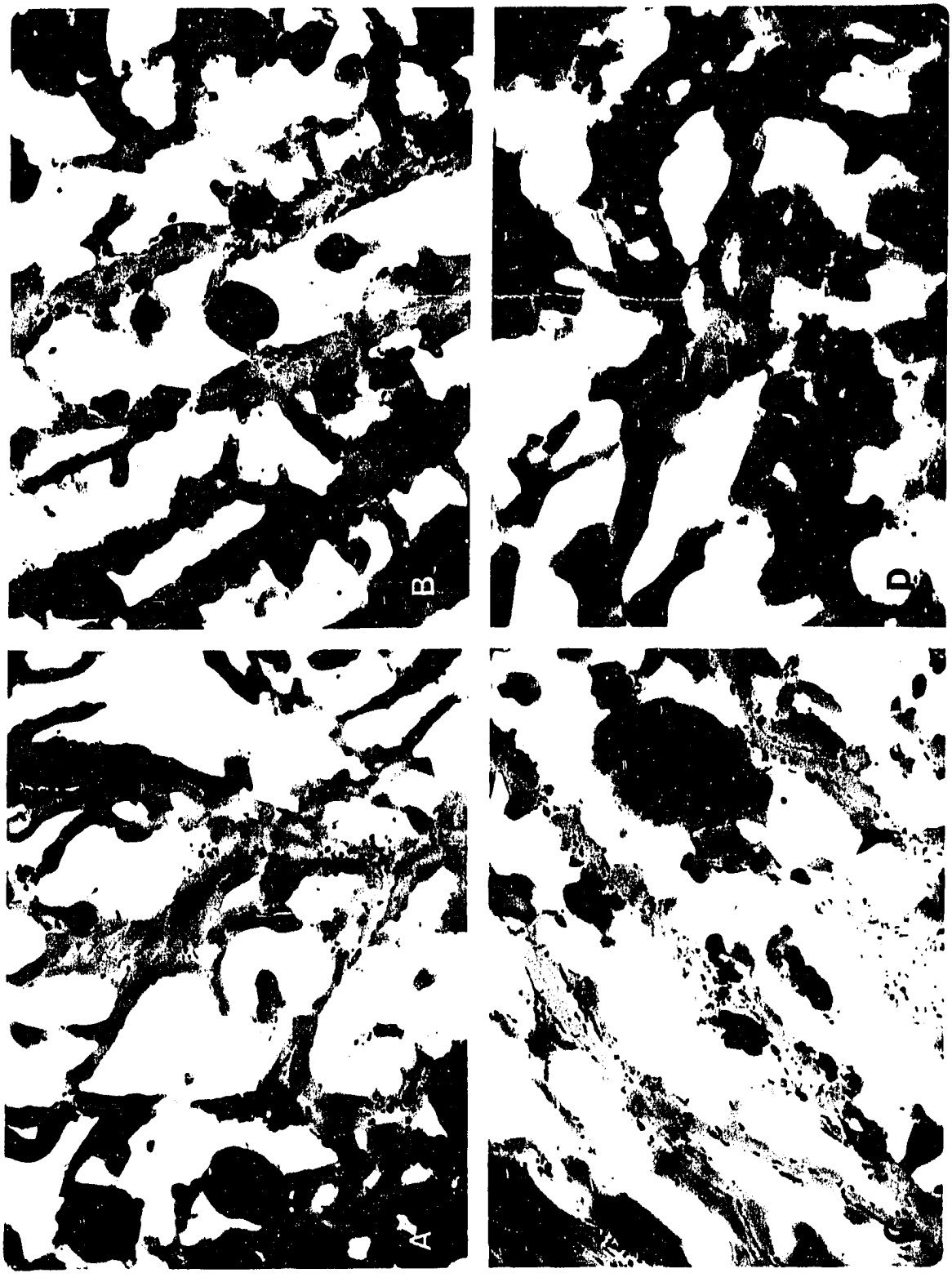

B
D
A

Figure 23. Light micrographs of ground beef patties frozen at an 80 minute freezing rate; stained with oil red 0 and hematoxylin. X 120

A) Formulation: 0% frozen-flaked trim

B) Formulation: 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation: 50% frozen-flaked trim, 50% nonfrozen trim

D) Formulation: 100% frozen-flaked trim

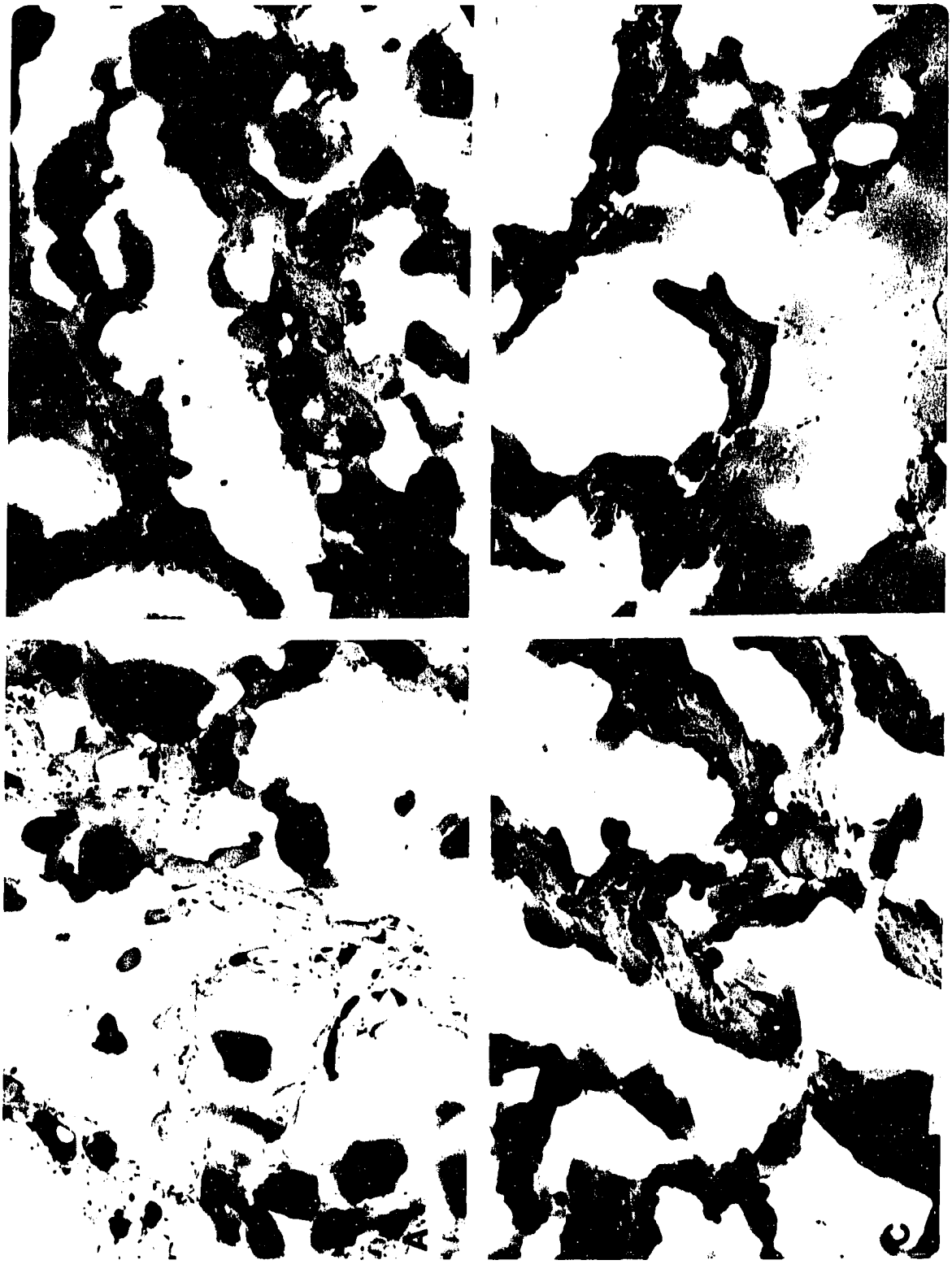


Figure 24. Light micrographs of ground beef patties frozen at a 100 minute freezing rate; stained with oil red 0 and hematoxylin. X 120

A) Formulation: 0% frozen-flaked trim

B) Formulation: 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation: 50% frozen-flaked trim, 50% nonfrozen trim

D) Formulation: 100% frozen-flaked trim

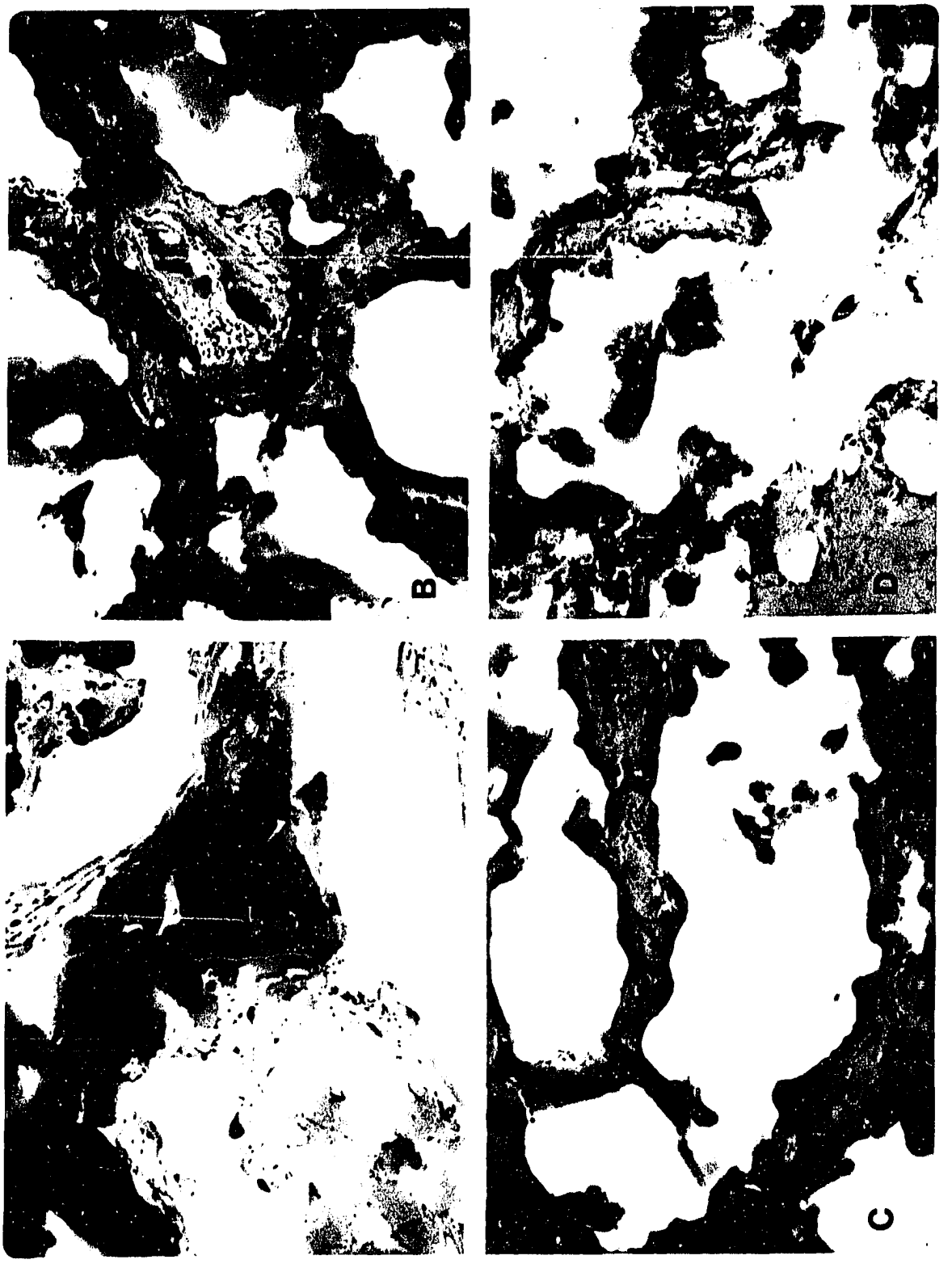

B
D
C

Figure 25. SEM micrographs of ground beef patties frozen at a 6 minute freezing rate; freeze-dried. X 120

A) Formulation: 0% frozen-flaked trim

B) Formulation: 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation: 50% frozen-flaked trim, 50% nonfrozen trim

D) Fromulation: 100% frozen-flaked trim

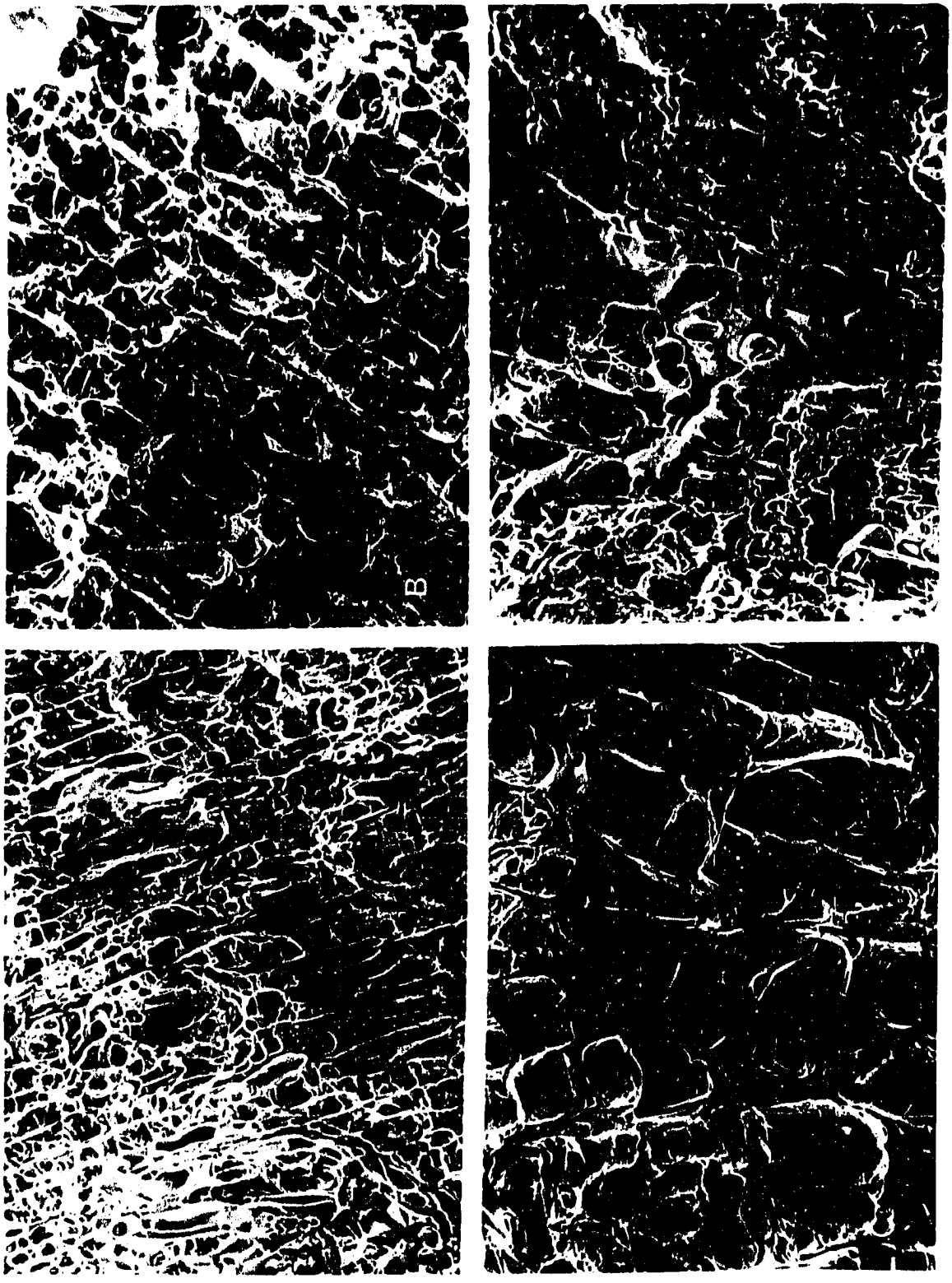

B

Figure 26. SEM micrographs of ground beef patties frozen at a 30 minute freezing rate; freeze-dried. X 120

A) Formulation 0% frozen-flaked trim

B) Formulation 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation 50% frozen-flaked trim, 50% nonfrozen trim

D) Formulation 100% frozen-flaked trim

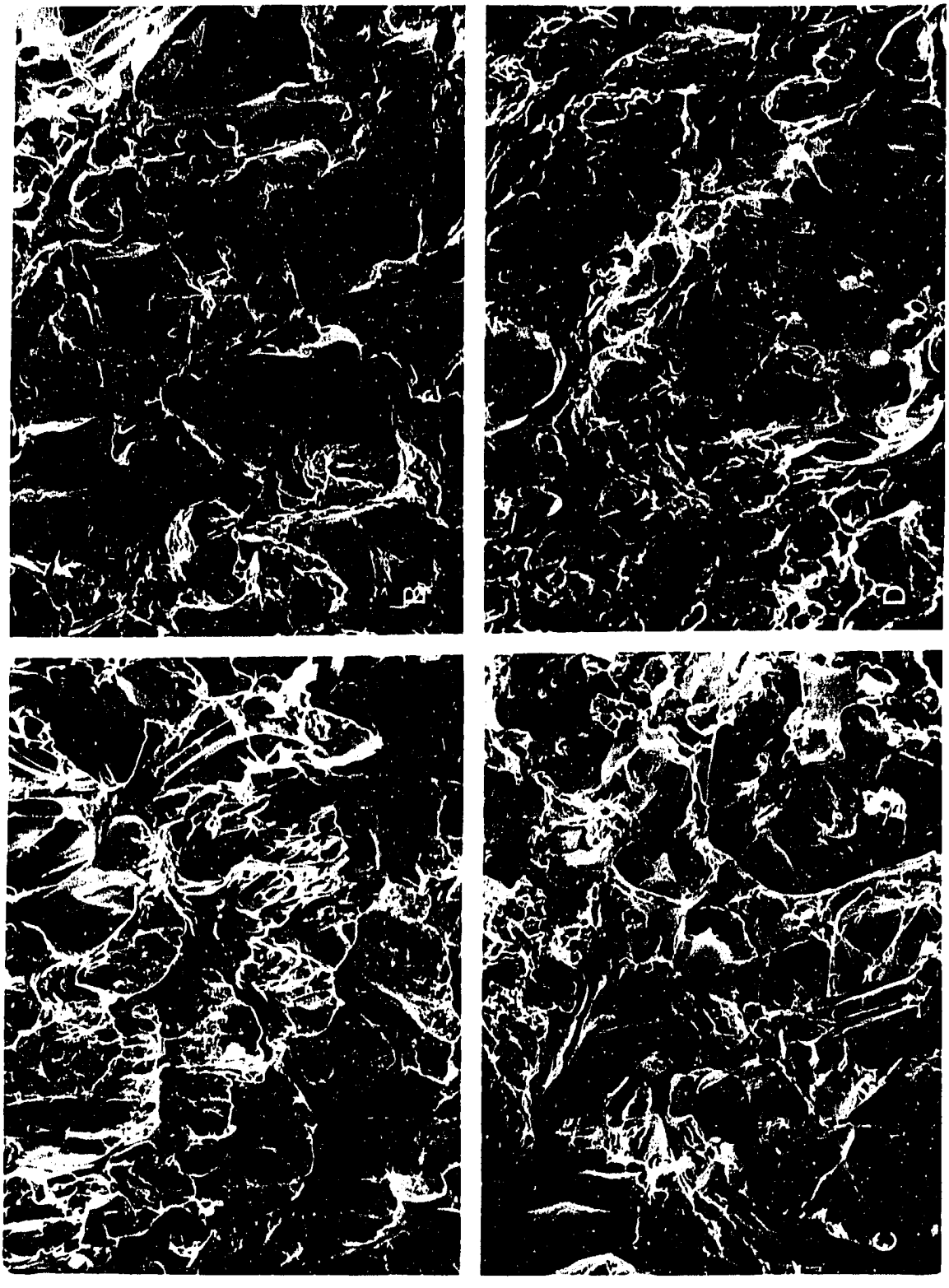


Figure 27. SEM micrographs of ground beef patties frozen at an 80 minute freezing rate; freeze-dried. X 120

A) Formulation: 0% frozen-flaked trim

B) Formulation: 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation: 50% frozen-flaked trim, 50% nonfrozen trim

D) Formulation: 100% frozen-flaked trim

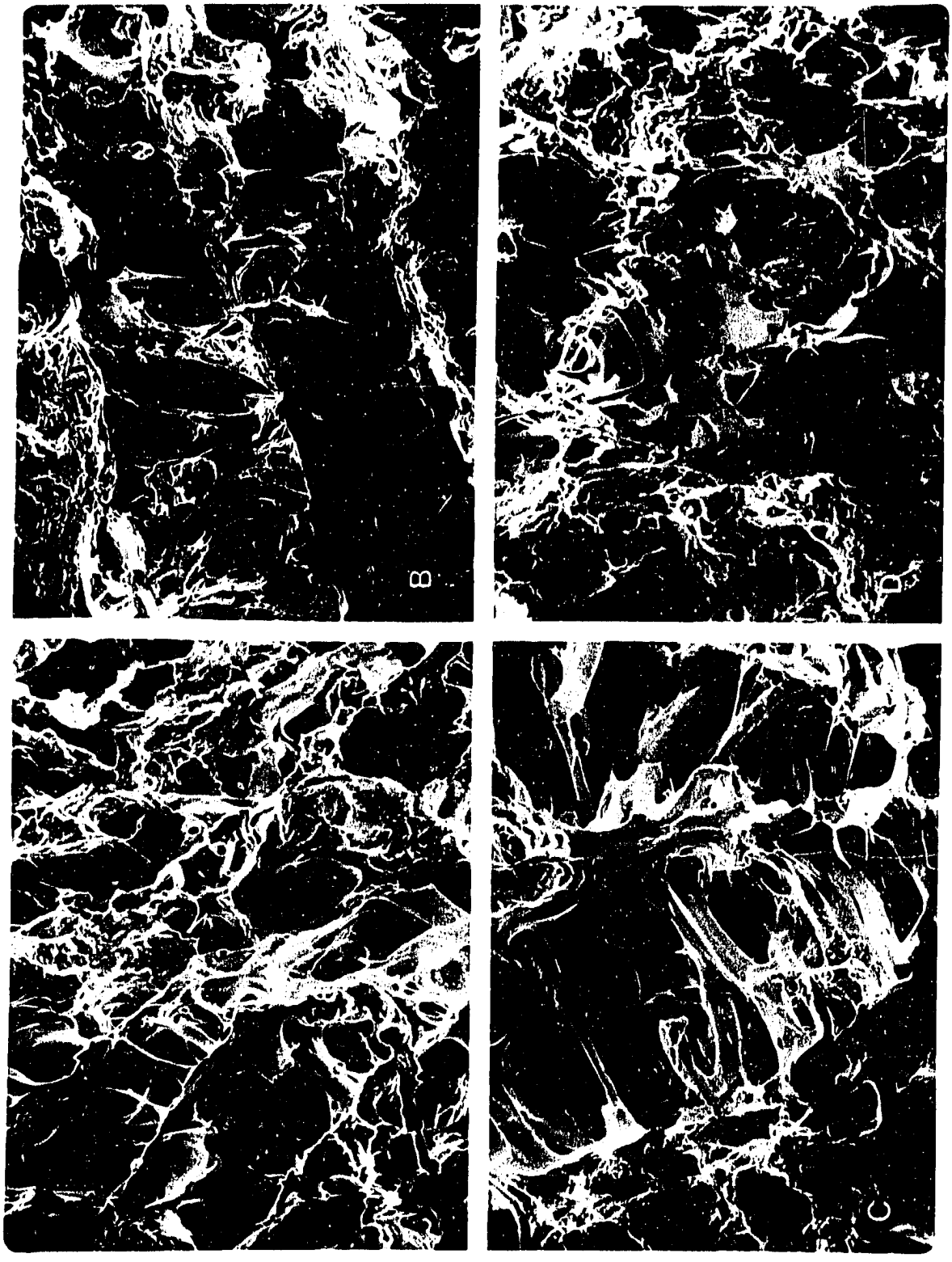

B
D
C

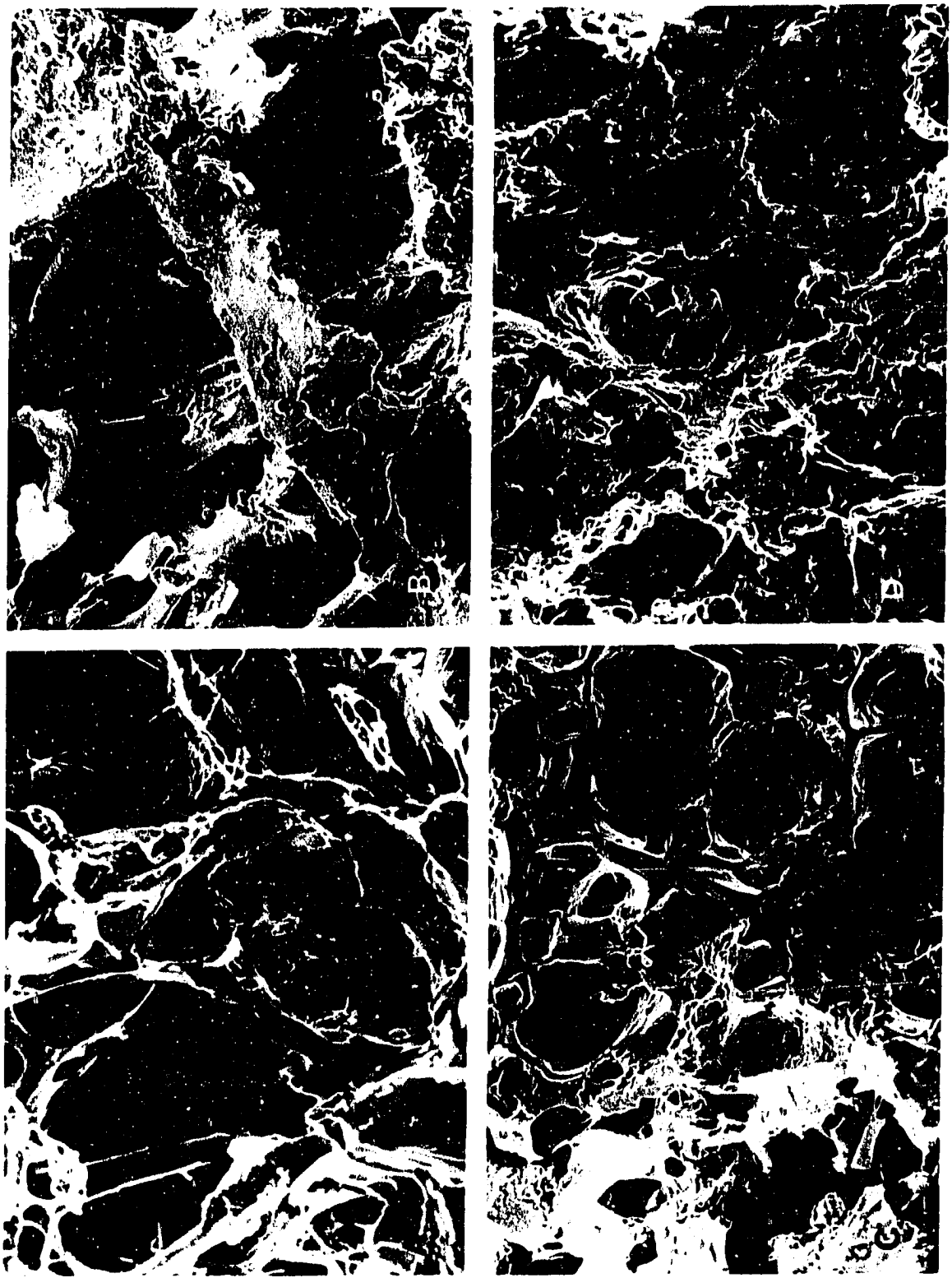
Figure 28. SEM micrographs of ground beef patties frozen at a 100 minute freezing rate; freeze-dried. X 120

A) Formulation: 0% frozen-flaked trim

B) Formulation: 20% frozen-flaked trim, 80% nonfrozen trim

C) Formulation: 50% frozen-flaked trim, 50% nonfrozen trim

D) Formulation: 100% frozen-flaked trim



The micrographs suggest that within a given freezing rate, all formulations react in a similar manner. However, at the 6 minute rate (Figures 21 and 25), both LM and SEM systems seem to show smaller diameter ice crystal cavities for the 0% frozen-flaked formulation. This observation was not evident at the other three freezing rates. Perhaps the tighter structure of the intact muscle fibers of the 0% frozen-flaked formulation, combined with the very rapid freezing rate, inhibits ice crystal growth. At slower freezing rates (Figures 27 and 28), SEM micrographs indicate that 100% frozen-flaked formulations withstand large ice crystal damage better than the other three formulations. The 100% formulation (D for all figures) also displays greater fiber fragmentation within each freezing rate which coincides with its consistently low shear values (Table 14 and Figure 5). Even though flaking improves tenderness (Table 14 and Figure 5), WHC (Table 3), and cook loss (Table 10 and Figure 4), it appears that formulations containing more than 50% frozen-flaked trim reduce flavor, juiciness and overall acceptability. Processors wanting to capitalize on the advantages of the 50 and 100% frozen-flaked formulations may improve quality by slightly modifying processing procedures. A longer mixing period after flaking may extract more soluble proteins resulting in a better bind among the flaked particles. Also, the addition of low levels of salt would

also extract more soluble proteins and similarly improve the cohesiveness of the pattie as well as possibly improving its flavor characteristics. Salt also would increase WHC (Hamm, 1977) which may considerably improve juiciness and, ultimately, overall pattie acceptability.

Unfrozen and cooked vs frozen and cooked comparisons (SEM)

Three unfrozen, cooked pattie formulations containing 0% frozen-flaked, 100% frozen-flaked and 100% prerigor trim and identical formulations cooked from the frozen state, after freezing at 6, 30, 80 and 100 minute rates, are shown in Figures 29-32. Micrographs of the 0% frozen-flaked and prerigor trim show longitudinal and cross-sectional examples of intact muscle fibers even after the cooking process (Figures 29, 30 and 32). The 100% frozen-flaked formulations (Figure 31) shows little evidence of fiber integrity and appears as a coagulated mass. This physical nature of the 100% frozen-flaked formulation, combined with its higher WHC (Table 3), may explain the significant decrease in cook loss during grilling.

A comparison of freezing rates within each cooked formulation (Figures 30, 31 and 32) indicate no structural differences or visible advantages for faster freezing rates as previously seen in precooked formulations (Figures 11-20). The cooking process also apparently masked the definite

Figure 29. SEM micrographs of unfrozen, cooked ground beef patties; freeze-dried

A) Formulation: 0% frozen-flaked trim; X 120

B) Formulation: 0% frozen-flaked trim; X 1200

C) Formulation: 100% frozen-flaked trim; X 120

D) Formulation: 100% prerigor trim; X 120

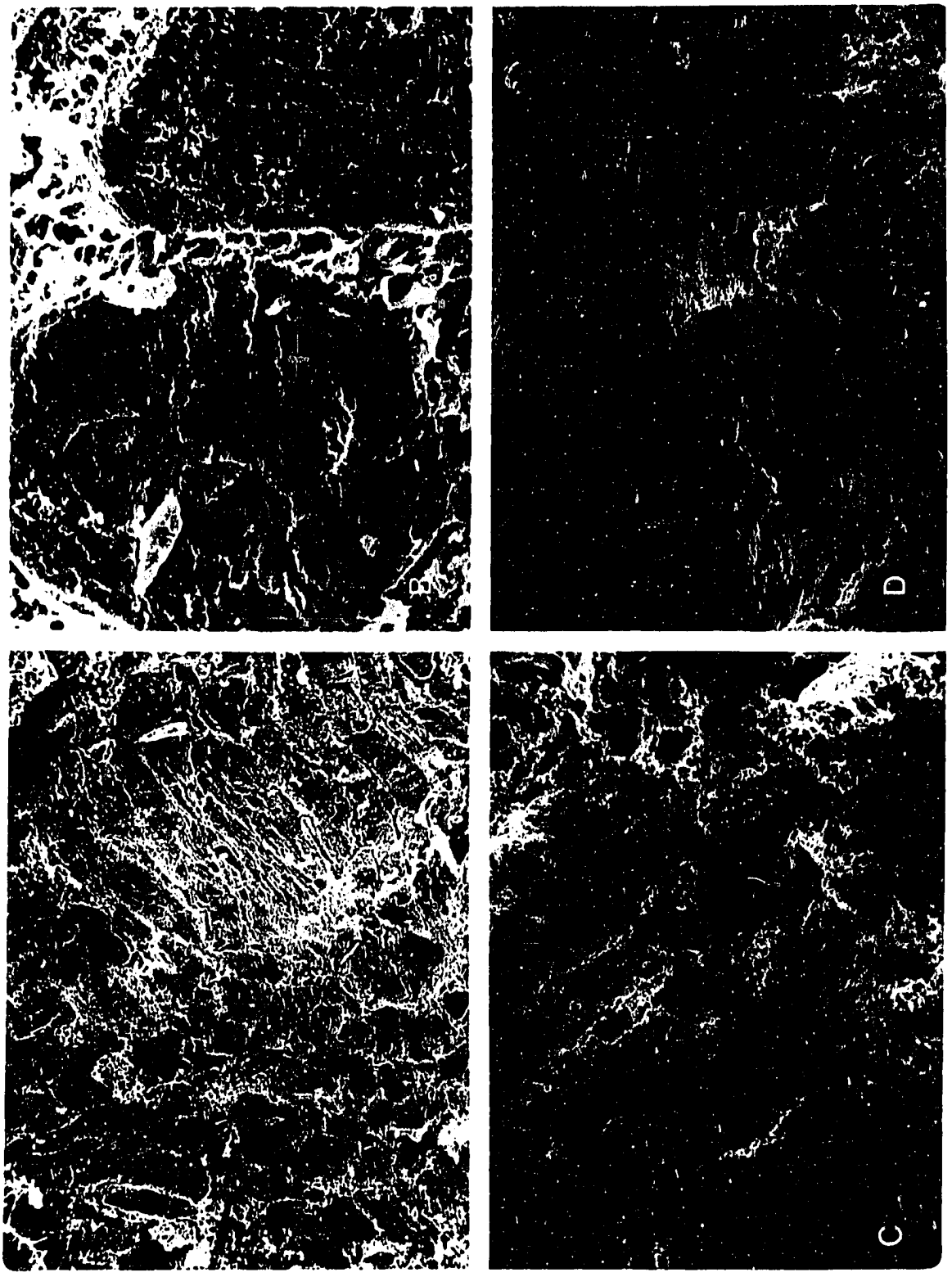

B
D
C

Figure 30. SEM micrographs of frozen, cooked ground beef patties formulated with 0% frozen-flaked trim; freeze-dried. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

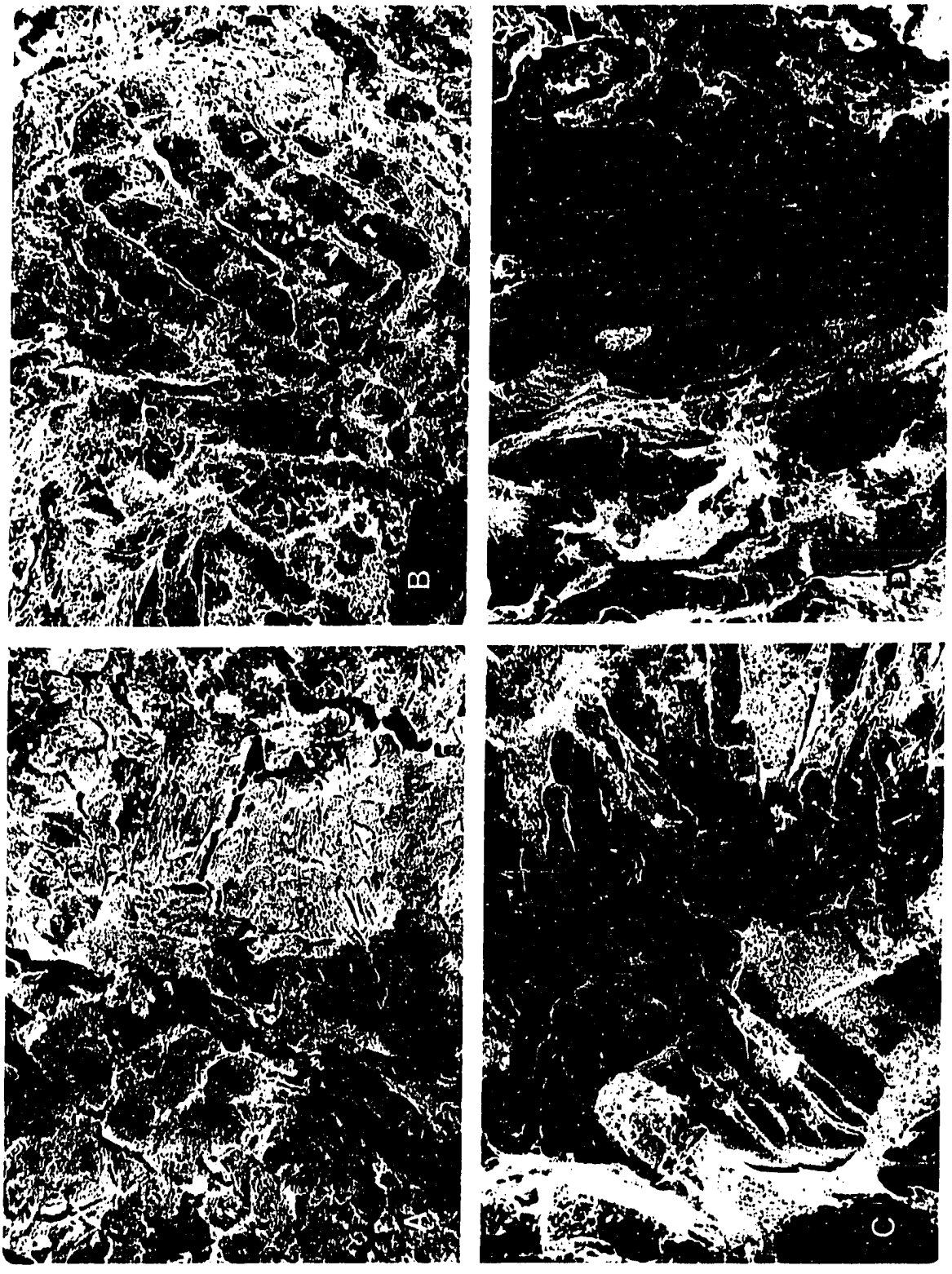

B
B
C

Figure 31. SEM micrographs of frozen, cooked ground beef patties formulated with 100% frozen-flaked trim; freeze-dried. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

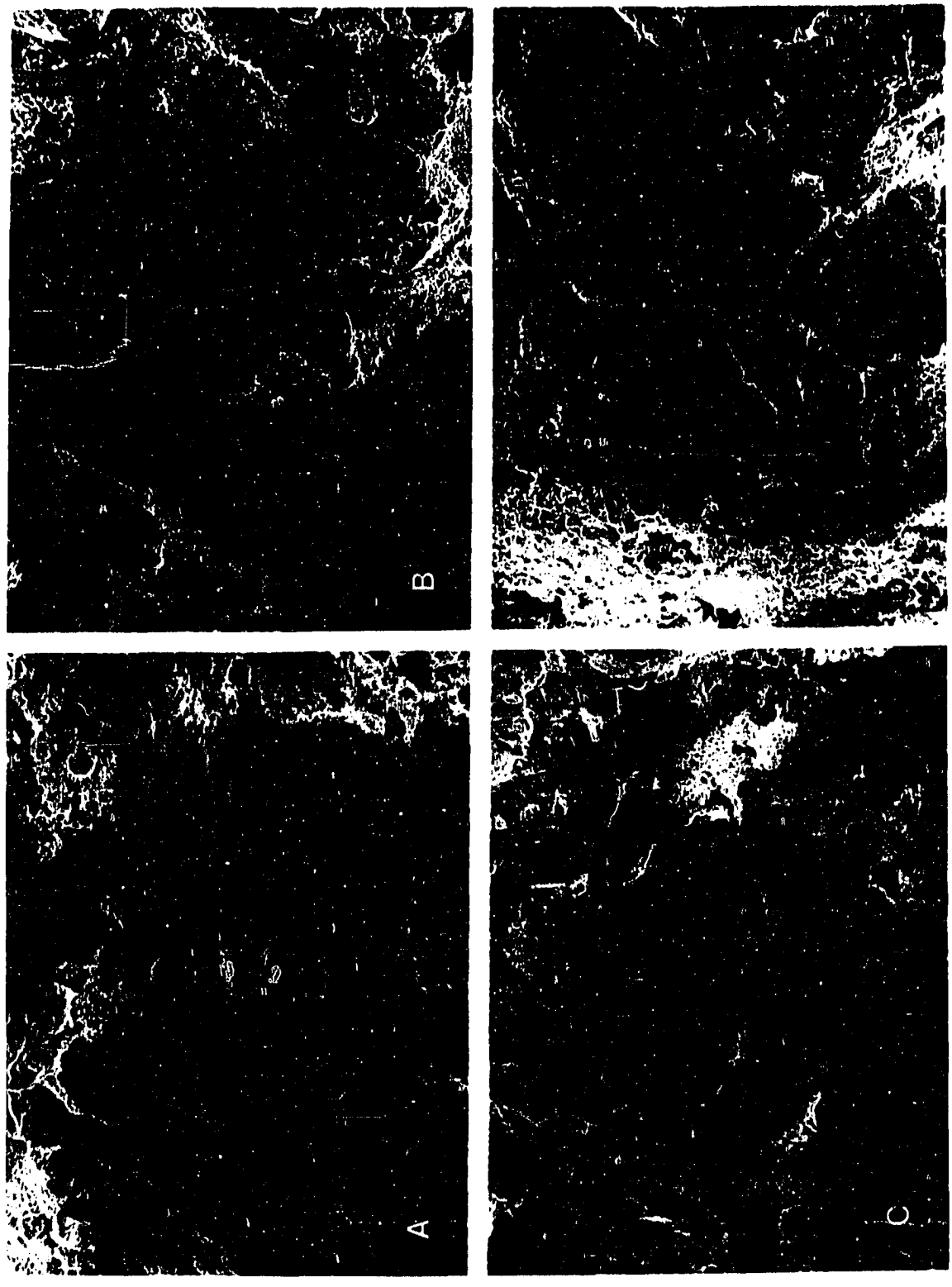

B
A
C

Figure 32. SEM micrographs of frozen, cooked ground beef patties formulated with 100% prerigor trim; freeze-dried. X 120

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes

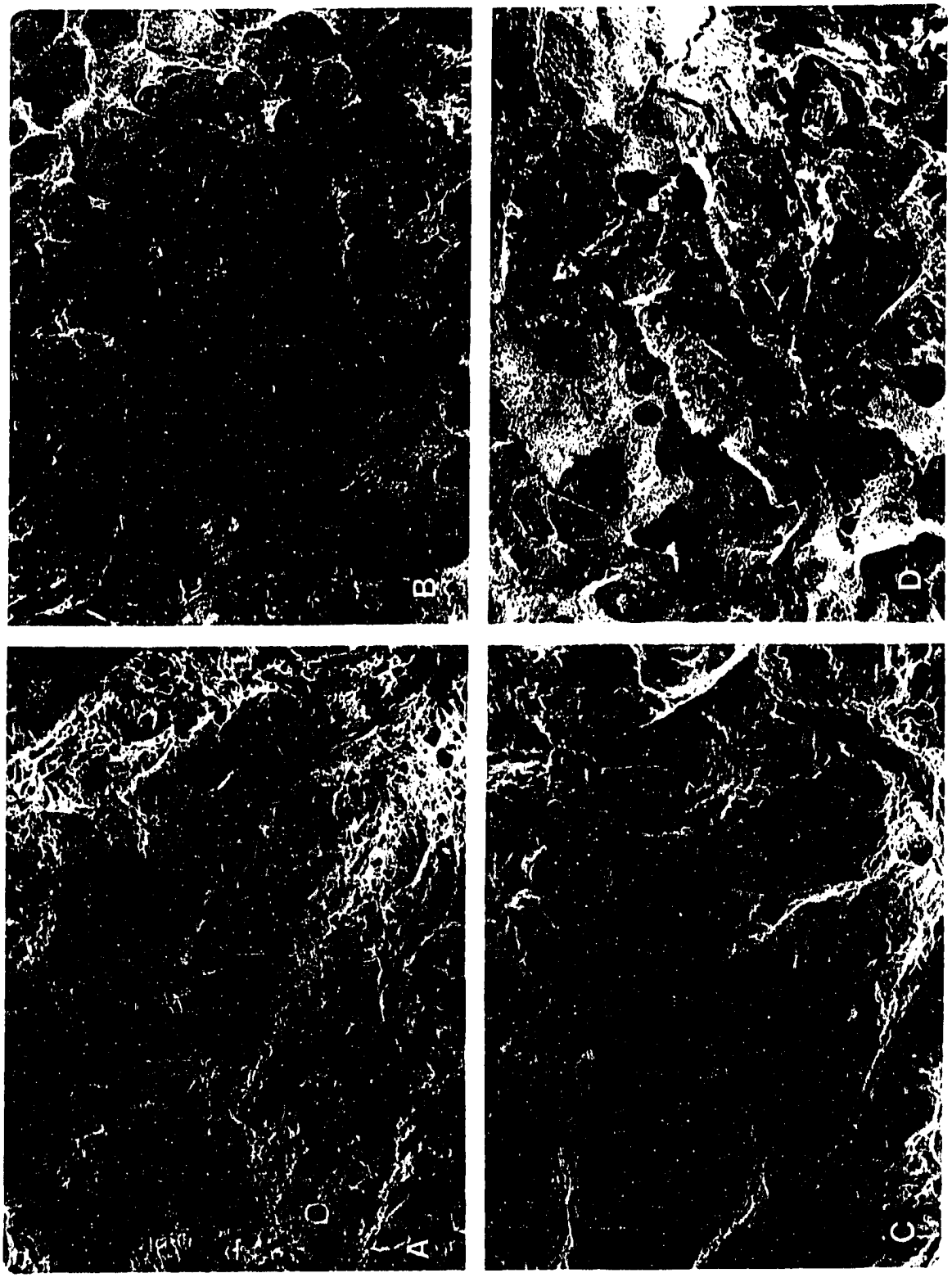

B
D
A
C

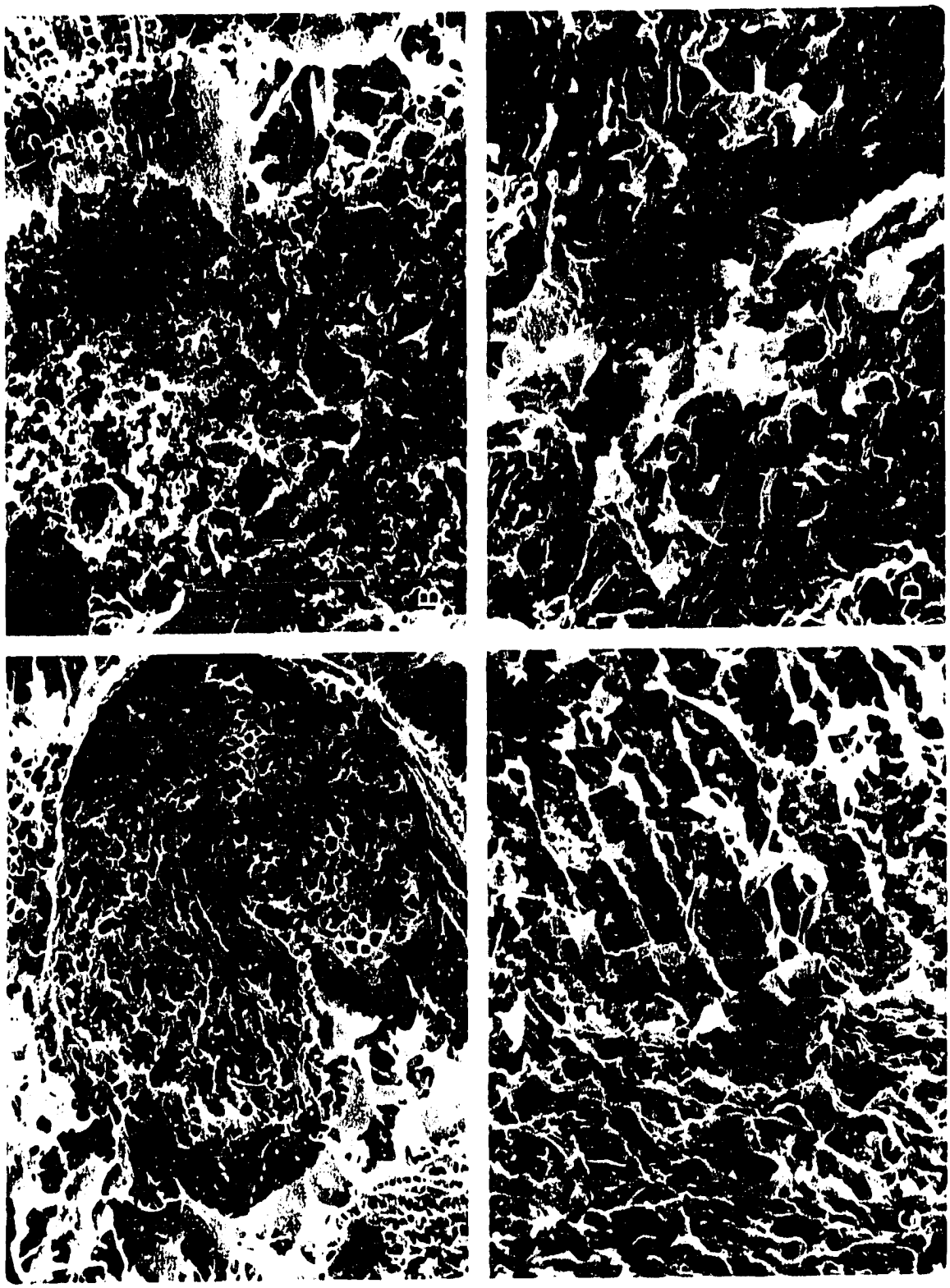
Figure 33. SEM micrographs of frozen, cooked ground beef patties formulated with 0% frozen-flaked trim; freeze-dried. X 1200

A) Freezing rate: 6 minutes

B) Freezing rate: 30 minutes

C) Freezing rate: 80 minutes

D) Freezing rate: 100 minutes



visible differences observed when comparing unfrozen and frozen precooked formulations. While no structural differences were apparent for the frozen, cooked, 0% frozen-flaked formulation (Figure 30) a similar comparison of the same formulation at a higher magnification (Figure 33) gives visible indication of smaller ice crystal cavities at the 6 minute freezing rate. Also, the ice cavities shown in Figure 33 were not present in the unfrozen, cooked, 0% frozen-flaked formulation (Figure 29,B), indicating that even after the cooking process, structural differences between unfrozen and frozen patties can sometimes be detected.

SUMMARY

Ground beef patties formulated with various combinations of frozen-flaked, nonfrozen and prerigor trim were frozen at different freezing rates and evaluated for freezing, thawing, cooking and sensory characteristics. Light and scanning electron micrographs of unfrozen, frozen and cooked pattie formulations were compared.

Fast freezing rates had a positive effect on improving frozen ground beef pattie quality. Reduced freezing time decreased freezing shrink loss and cooking loss while increasing tenderness, juiciness and overall acceptability scores. The photomicrographs indicate greater ice crystal damage at slower rates of freezing. The micrographs also indicate that structural differences caused by various freezing rates were generally eliminated by cooking.

The addition of up to 50% frozen-flaked trim to the 0% frozen-flaked pattie formulation was essentially undetectable and had no adverse effect on pattie quality traits. Pattie formulations containing greater than 50% frozen-flaked trim had lower cooking loss, greater WHC and higher tenderness scores than patties with lower percentages of frozen-flaked trim. However, sensory scores for flavor, juiciness and overall acceptability were less desirable. Patties formulated from prerigor trim compared favorably with normal postrigor patties in all quality tests.

CONCLUSIONS

Freezing rates of up to 80 minutes can be utilized for producing frozen ground beef patties without sacrificing flavor, tenderness, juiciness or overall acceptability. However, faster freezing rates significantly reduce freezer shrink and should be utilized when economically feasible.

Formulations containing 20 or 50% frozen-flaked trim have acceptable quality and can be used successfully for frozen ground beef patties. Increasing the amount of frozen-flaked trim beyond 50% of the formulation adversely affects pattie quality even though tenderness is increased and cooking loss is reduced.

Prerigor trim, when processed correctly, can be successfully utilized for frozen ground beef patties.

Structural differences caused by freezing rate or pattie formulation can be successfully related to several pattie quality characteristics with LM and SEM.

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BIBLIOGRAPHY

AMI. 1978. Meat facts. American Meat Institute, Washington, D.C.

AOAC. 1965. Official Methods of Analysis, 10th ed. Association of Official Analytical Chemists, Washington, D.C.

AOAC. 1975. Official Methods of Analysis, 12th ed. Association of Official Analytical Chemists, Washington, D.C.

Ary, J. and B. B. McLean. 1946. Thawing and cooking lamb legs. J. Home Econ. 36:646.

Ashby, B. H., G. M. James and A. Kramer. 1973. Effects of freezing and packaging methods on shrinkage of hams in frozen storage. J. Food Sci. 38:254.

Aström, S. 1971. Freezing equipment influence on weight losses. In F. Stewart and C. Willey eds. Proceedings of the Third International Congress of Food Science and Technology. Institute of Food Technologists, Chicago.

Bannister, M. A., D. L. Harrison, A. D. Dayton, D. J. Kropf and H. J. Tuma. 1971. Effects of a cryogenic and three home freezing methods on selected characteristics of pork loin chops. J. Food Sci. 36:951.

Barr, A. J., J. H. Goodnight, J. P. Sall and J. T. Helvig. 1976. A users guide to the statistical analysis system. North Carolina State University Press, Raleigh, N.C.

Bate-Smith, E. C. and J. R. Bendall. 1949. Factors determining the time course of rigor mortis. J. Physiol. 110:47.

Behnke, J. R. 1976. Freezing. Food Technol. 30(12):32.

Bendall, J. R. 1960. Postmortem changes in muscle. Page 227 in G. H. Bourne, ed. The structure and function of muscle. Academic Press, New York.

Berry, B. W., G. C. Smith, J. V. Spencer and G. H. Kroening. 1971. Effects of freezing method, length of frozen storage and cookery from the thawed or frozen state on palatability characteristics of pork. J. Anim. Sci. 32:636.

Birkner, M. L. and E. Auerbach. 1960. The Science of Meat and Meat Products. American Meat Institute Foundation, W. H. Freeman and Co., San Francisco.

Boyd, A. 1978. Pros and cons of critical point drying and freeze drying for SEM. Scanning Electron Microscopy 2:303.

Brady, D. E., P. Frei and C. W. Hickman. 1942. Effect of freezing rate on quality of broiled steaks. Food Res. 7:388.

Bratzler, L. J. 1949. Determining the tenderness of meat by use of the Warner-Bratzler method. Proc. Recip. Meat Conf. 1949:117.

Briskey, E. J., W. G. Hoekstra, R. W. Bray and R. H. Grummer. 1960. A comparison of certain physical and chemical characteristics of eight pork muscles. J. Anim. Sci. 19:214.

Bucholz, S. B. and G. M. Pigott. 1972. Immersion freezing of fish in dichlorodifluoromethane. J. Food Sci. 37:416.

Callow, E. H. 1952. Frozen meat. J. Sci. Food Agric. 3:145.

Cassens, R. G. and R. P. Newbold. 1967. Temperature dependence of pH changes in ox muscle postmortem. J. Food Sci. 32:13.

Causey, K., M. E. Hausrath, P. E. Raustad and F. Fexitoxi. 1950. Effect of thawing and cooking methods on palatability and nutritive value of frozen ground meat. III. Lamb. Food Res. 15:256.

Chambers, R. and H. P. Hale. 1932. The formation of ice in protoplasm. Proc. Roy. Soc. (London) Ser. B. 110:336.

Child, A. M. and P. Paul. 1937. Effect of thawing and cooking frozen pork and beef. Minn. Agr. Exp. Sta. Bul. 125.

Cook, G. A., E. F. J. Love, J. R. Vickery and W. J. Young. 1926. Studies on the Refrigeration of Meat. Australian J Exp. Biol. Med. Sci. 3:15.

Cover, S. and R. L. Hostetler. 1960. Beef tenderness by new methods. Tex. Agr. Exp. St. Bull. 947.

Cutting, C. L. and R. Malton. 1974. Evaporative losses in the commercial freezing and frozen storage of meat. Proc. Br. Meat Res. Inst. Symp. 1974:366.

Dawson, E. H., G. S. Linton, A. M. Harkin and C. Miller. 1959. Factors influencing the palatability, vitamin content, and yield of cooked beef. Home Econ. Res. Report No. 9:1.

Deatherage, F. E. and R. Hamm. 1960. Influence of freezing and thawing on hydration and charges of the muscle proteins. Food Res. 25:623.

Desrosier, N. W. 1970. The technology of food preservation. 3rd ed. AVI Publishing Co., Inc., Westport, Conn.

Dubois, C. W. and D. K. Tressler. 1940. Influence of rate of freezing and temperature of storage on quality of frozen meat. Proceedings of the First Food Conference. Inst. Food Technologists 1940:167.

Dubois, C. W., D. K. Tressler and F. Fenton. 1942. The effect of the rate of freezing and temperature of storage on the quality of frozen poultry. Refrig. Eng. 44:93.

Dunker, C. F. and O. G. Hankins. 1953. Rates of freezing and thawing meats. Food Technol. 7:505.

Empey, W. A. 1933. Studies on the refrigeration of meat. Conditions determining the amount of "drip" from frozen and thawed muscle. J. Soc. Chem. Ind. 52:230T.

Fennema, O. 1968. General principles of cryogenic processing. Proc. Meat Ind. Res. Conf. 1968:109.

Fennema, O. and W. D. Powrie. 1964. Fundamentals of low-temperature food preservation. Adv. Food Res. 13:219.

Footrakul, P. 1976. Freezing and thawing effects on the quality and heat transfer characteristics of ground beef. Ph.D. thesis. University of Massachusetts, Amherst, Mass.

Gensho, I. 1950. Freezing characteristics of beef. Japan Sci. Rev. 1:71.

Grau, R. and R. Hamm. 1953. A simple method for the determination of water-binding in muscles. Naturwissenschaften 40:29.

Gray, R. 1967. Red meat commercially frozen with liquid nitrogen system. Quick Frozen Foods 29(7):129.

Guenther, J. J. and R. L. Henrickson. 1962. Temperatures, methods used in freezing determine tenderness, color of meat. Quick Frozen Foods 25:115.

Hamm, R. 1959. Biochemistry of meat hydration. Am. Meat Inst. Found. Res. Conf. Proc. 11:17.

Hamm, R. 1960. Biochemistry of meat hydration. Adv. Food Res. 10:355.

Hamm, R. 1977. Postmortem breakdown of ATP and glycogen in ground muscle: A review. Meat Sci. 1:15.

Hankins, O. G. and R. L. Hiner. 1940. Freezing makes beef tenderer. Food Ind. 12:49.

Heldman, D. R. 1977. Food Process Engineering. AVI Publishing Co., Westport, Conn.

Hiner, R. L. and O. G. Hankins. 1946. Fiber splitting results in more tender beef. Quick Frozen Foods 8:115.

Hiner, R. L. and O. G. Hankins. 1947. Temperature of freezing affects tenderness of beef. Food Ind. 19:1078.

Hiner, R. L. and A. G. Hankins. 1951. Effect of freezing of beef from different muscles and from animals of different ages. Food Technol. 5:374.

Hiner, R. L., L. L. Madsen and O. G. Hankins. 1945. Histological characteristics, tenderness, and drip losses of beef in relation to temperature of freezing. Food Res. 10:312.

Humason, G. L. 1972. Animal Tissue Techniques. W. H. Freeman and Co., San Francisco.

Huxley, H. E. 1953. Electron microscope studies of the organization of the filaments in striated muscle. Biochim. Biophys. Acta 12:387.

Huxley, H. E. 1969. The mechanisms of muscular contraction. Science 164:1356.

Jakobsson, B. and N. Bengtsson. 1973. Freezing of raw beef: Influence of aging, freezing rate and cooking method on quality and yield. J. Food Sci. 38:560.

Jensen, W. A. 1954. The application of freeze-dry methods to plant material. Stain Technol. 29(3):143.

Judge, M. D. 1974. Meat technology to meet the needs of the food service industry: Meat patties. Proc. Recip. Meat Conf. 27:396.

Karow, A. M., Jr. and W. R. Webb. 1965. Tissue freezing. A theory for injury and survival. Cryobiology 2(3):99.

Kassai, D. 1970. Thawing of frozen carcass. Food Sci. Tech. Abstr. 2(7):S528.

Kastner, C. L. and R. L. Henrickson. 1969. Providing uniform meat cores for mechnical shear force measurements. J. Food Sci. 34:603.

Koonz, C. H. and J. M. Ramsbottom. 1939. A method for studying the histological structure of frozen products. I. Poultry. Food Res. 4:117.

Koreshkov, V. N., V. V. Guslyannikou and G. G. Tinyakov. 1975. Biological structure of poultry meat frozen in liquid nitrogen and in air. Bulletin De L'institut International Du Froid 247.

LaSalle, B. 1975. Cryogenic preservation of live cells. Round Table Conference on the Cryogenic Preservation of Cell Cultures. National Academy of Sciences, Washington, D.C.

Law, H. M., S. P. Yang, A. M. Mullins and M. M. Fielder. 1967. Effect of storage and cooking on qualities of loin and top-round steaks. J. Food Sci. 32:637.

Lin, H. 1976. The influence of prerigor and postrigor processing on the shelf-life of fresh pork sausage. M.S. Thesis. Iowa State University, Ames, Iowa.

Lind, M. L., D. L. Harrison and D. H. Kropf. 1971. Freezing and thawing rates of lamb chops: Effects on palatability and related characteristics. J. Food Sci. 36:629.

Locker, R. H. and C. J. Hagyard. 1963. A cold shortening effect in beef muscles. J. Sci. Food Agric. 14:787.

Love, R. M. 1962. Protein denaturation in frozen fish. VI. Cold storage studies on cod using the cell fragility method. J. Sci. Food Agr. 13:269.

Love, R. M. 1966. The freezing of animal tissue. Pages 317-398 in H. T. Meryman, ed. Cryobiology. Academic Press, New York.

Love, R. M. and S. B. Haraldsson. 1961. The expressible fluid of fish fillets. XI. Ice crystal formation and cell damage in cod muscle frozen before rigor mortis. J. Sci. Food Agr. 12:442.

McClain, P. E. and A. M. Mullins. 1969. Relationship of water-binding capacity and pH to tenderness of bovine muscles. J. Anim. Sci. 29(2):268.

MacDougall, D. B. 1974. The appearance of frozen meat and its color stability during storage. Proc. Br. Meat Res. Inst. Symp., p. 103.

MacKay, D. D. and C. B. Weld. 1926. Characteristics of frozen fish fillets. Rep. Biol. Bd. Can. No. 15.

Marsh, B. B. and J. F. Thompson. 1957. Thaw rigor and delta state of muscle. Biochim. Biophys. Acta 24:427.

Marsh, B. B. and J. F. Thompson. 1958. Rigor mortis and thaw rigor in lamb. J. Sci. Food Agr. 9:417.

Menz, L. J. and B. J. Luyet. 1961. An electron microscope study of the distribution of ice in single muscle fibers frozen rapidly. Biodynamica 8:261.

Meryman, H. T. 1956. Mechanics of freezing in living cells and tissues. Science 124:515.

Meryman, H. T. 1963. Preservation of living cells. Federation Proceedings 22:81.

Miller, W. D., R. L. Saffle and S. B. Zirkle. 1968. Factors which influence the water-holding capacity of various types of meat. Food Technol. 22(9):1139.

Moran, T. 1930. Critical temperature of freezing-living muscle. Proc. Roy. Soc. (London) 105B:177.

Moran, T. 1932. Rapid freezing. Critical rate of cooling. J. Soc. Chem. Ind. 51:16T.

Moran, T. And H. P. Hale. 1932. Rapid freezing. Temperature of storage. J. Soc. Chem. Ind. 51:20T.

Morley, M. J. 1974. Thermophysical properties of frozen meat. Proc. Br. Meat Res. Inst. Symp. 1974:131.

Munro, H. N. and A. Fleck. 1966. The determination of nucleic acids. Methods Biochem. Anal. 14:113.

Ockerman, H. W. 1974. Quality Control of Post-Mortem Muscle Tissue. 9th ed. Ohio State University and Ohio Agricultural Research and Development Center.

Paul, P. and A. M. Child. 1937. Effect of freezing and thawing beef muscle upon press fluid, losses and tenderness. Food Res. 2:339.

Pearson, A. M. and J. I. Miller. 1950. The influence of rate of freezing and length of freezer-storage upon the quality of beef of known origin. J. Anim. Sci. 9:13.

Penny, J. F. 1974. The effect of freezing on the amount of "drip" from meat. Meat Res. Inst. Symp. No. 3:8.1.

Pepe, F. A. 1971. Structure of the myosin filament of striated muscle. Prog. in Biophys. Molec. Biol. 22:77.

Ramsbottom, J. M. 1947. Freezer storage effect on fresh meat quality. Refrig. Eng. 53:19.

Ramsbottom, J. M. and C. H. Koonz. 1939. Freezing temperature as related to drip of frozen-defrosted beef. Food Res. 4:425.

Ramsbottom, J. M. and C. H. Koonz. 1940. Relationship between time of freezing beef after slaughter and amount of drip. Food Res. 5:423.

Ramsbottom, J. M. and C. H. Koonz. 1941. Freezer storage temperature as related to drip and to color in frozen-defrosted beef. Food Res. 6:571.

Rapatz, G. and B. Luyet. 1959. On the mechanism of ice formation and propagation in muscle. Biodynamica 8:122.

Rasmussen, C. L. and R. L. Olson. 1972. Freezing methods as related to cost and quality. Food Technol. 26:32.

Reay, G. A. 1934. The influence of freezing temperature on haddock's muscle. II. J. Soc. Chem. Ind. 53:413T.

Richardson, W. D, and E. Scherubel. 1908. General introduction and experiments on frozen beef. J. Am. Chem. Soc. 30, 1515.

Ritchey, S. J. and R. L. Hostetler. 1964. Relationships of free and bound water to subjective scores for juiciness and softness and to changes in weight and dimensions of steaks from two beef muscles during cooking. J. Food Sci. 29:413.

Root, L. 1978. McDonald's: serving up 9 million pounds weekly in hamburger trade. Drovers Journal Roll Call 32:64.

Ryan, J. P. 1966. Liquid nitrogen freezing of poultry products. Quick Frozen Foods 28(12):99.

Sair, L. and W. H. Cook. 1938. Relation of pH to drip formation in meat. Can. J. Res. 16D:255.

Sang, N. P. 1977. Effect of cryogenic and blast freezing on quality traits of ground beef patties. M.S. Thesis. Iowa State University, Ames, Iowa.

Schmidt, G. and S. J. Thannhauser. 1945. A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. J. Biol. Chem. 161:83.

Scopes, R. K. 1970. Characterization and study of sarcoplasmic proteins. Pages 471-492 in E. J. Briskey, R. G. Cassens and B. B. Marsh, eds. Physiology and Biochemistry of Muscle as a food. University of Wisconsin Press, Madison, Wis.

Sebranek, J. G. 1977. Organoleptic influences of cryogenic freezing for ground beef patties. Proceedings I. S. U. Meat Science Seminar Extension Bull. EC-1249.

Sebranek, J. G., P. N. Sang, R. E. Rust, D. G. Topel, and A. A. Kraft. 1978. Influence of liquid nitrogen, liquid carbon dioxide and mechanical freezing on sensory properties of ground beef patties. J. Food Sci. 43:842.

Smith, P. 1970. Future in cryogenic preservation of meat and meat products. Presented at Second Annual Future for Frozens Conf., Nov. 5, 1970 at Ames, IA. Dept. of An. Sci., Iowa State University.

Smith, G. C., C. W. Spaeth, Z. L. Carpenter, G. T. King, and K. E. Hoke. 1968. The effects of freezing, frozen storage conditions and degree of doneness on lamb palatability characteristics. J. Food Sci. 33:19.

Smith, G. C., Z. L. Carpenter and G. T. King. 1969. Consideration for beef tenderness evaluations. J. Food Sci. 34:612.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods. 6th ed. Iowa State University Press, Ames, Iowa.

Snyder, H. E. 1968. The study of myoglobin derivatives in meat samples by reflectance spectrophotometry. Proceedings Meat Ind. Res. Conf., p. 21. Am. Meat Inst. Found., Chicago, Il.

Sulzbacher, W. L. and A. M. Gaddis. 1968. Meats: Preservation of quality by freezer storage. In the Freezing Preservation of Foods, Vol. 2. AVI Publishing Co. Westport, Conn.

Squire, J. M. 1973. General model of myosin filament structure. 111. Molecular packing arrangements in myosin filaments. J. Mol. Biol. 77:291.

Swift, C. E. and M. D. Berman. 1959. Factors affecting the water retention of beef. I. Variations in composition and properties among eight muscles. Food Technol. 13:365.

Taylor, H. F. 1930. Solving problems of rapid freezing. Food Ind. 2:146.

Taylor, H. F. 1931. What happens during quick freezing. Food Ind. 3:205.

Trenkle, A., D. L. DeWitt and D. G. Topel. 1978. Influence of age, nutrition and genotype on carcass traits and cellular development of M. longissimus of cattle. J. Anim. Sci. 46:1597.

Tuma, H. J. 1971. Processing technology for freezing retail meat cuts. Proc. Meat Ind. Res. Conf., Am. Meat Inst. Found., Chicago, Il.

Voyle, C. A. 1974. Structure and histological changes associated with the freezing of meat. Meat Res. Inst. No. 3:6.1.

Wang, H., E. Auerbach, V. Bates, D. M. Doty and H. R. Kraybill. 1954. A histological and histochemical study of beef dehydration. Food Res. 19:543.

Weber, A. and J. M. Murray. 1973. Molecular control mechanisms in muscle contraction. Physiol. Rev. 53:612.

Weiner, P. D. 1964. Effect of processing pork carcasses prior to rigor mortis upon muscle and fat quality. M. S. Thesis. Kansas State University, Manhattan, Kansas.

Weir, C. E. 1960. The science of meat and meat products. American Meat Institute Foundation, W. H. Freeman and Co., San Francisco.

Wierbicki, E., L. E. Kunkle, and F. E. Deatherage. 1957a. Changes in the water-holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinkage. Food Technol. 11:69.

Wierbicki, E., V. R. Cahill and F. E. Deatherage, 1957b. Effects of added sodium chloride, calcium chloride, magnesium chloride, and citric acid on meat shrinkage at 70"C and of added sodium chloride on drip losses after freezing and thawing. Food Technol. 11:74.

Woolrich, W. R. 1948. Freezing damage in shrimp. Proc. Inst. Refrig. 45:41.

APPENDIX A

Table 19. Correlations among variables studied

	% Shrink	Color score	% Thaw gain	% Cook loss	pH	% Drip loss
Shrink, %	1.00					
Color score	-0.80**	1.00				
Thaw gain, %	-0.48**	0.48**	1.00			
Cook loss, %	-0.02	-0.11	-0.08	1.00		
pH	0.50**	-0.09	-0.33*	0.50**	1.00	
Drip loss, %	-0.43**	0.44**	0.34**	-0.41**	-0.33**	1.00
DNA (mg/ml)	0.31**	-0.51**	-0.21*	0.12	-0.19	-0.39**
Protein (mg/ml)	-0.27**	0.00	0.13	-0.02	-0.58**	0.19*
Shear value	0.18**	-0.46**	-0.21	0.18**	0.10	-0.54**
Flavor score	-0.06	0.09	-0.01	-0.02	0.03	-0.18**
Texture score	-0.28**	0.25**	0.17**	-0.12	-0.12	0.00
Juiciness score	-0.26**	-0.07	0.04	0.07	0.35**	-0.17*
Overall acceptability score	-0.21**	0.12	0.06	-0.03	-0.03	-0.11

*$p<0.05$; **$p<0.01$

(mg/ml) DNA	(mg/ml) Protein	Shear value	Flavor score	Texture score	Juici-ness score	Overall accepta-bility score
1.00						
0.19*	1.00					
0.53**	0.11	1.00				
0.01	0.02	0.14**	1.00			
0.00	0.00	-0.19**	0.28**	1.00		
0.01	0.02	0.11**	0.37**	0.54**	1.00	
-0.11	0.16	0.09*	0.72**	0.51**	0.59**	1.00

Table 20. Main effect treatment means[1] for total DNA content[2] of pattie drip

Formulation	Total DNA, mg	Freezing rate	Total DNA, mg
0% Frozen-flaked trim	0.724[c]	6 min. rate	0.856[a,b]
20% Frozen-flaked trim	0.518[d]	30 min. rate	0.829[a,b]
50% Frozen-flaked trim	0.712[c]	80 min. rate	0.953[a]
100% Frozen-flaked trim	0.916[b]	100 min. rate	0.741[b]
100% Prerigor trim	1.023[a,b]		
100% Postrigor trim	1.176[a]		

[1]Treatment means in a column with the same superscripts are not significantly different ($p<0.05$).

Table 21. Mean squares of the analysis of variance for percentage freezing shrink and percentage weight gain and cooking loss after thawing at 5°C and 20°C

Source	d.f.	% Shrink	% Wt. gain, 5°C thaw	% Wt. gain, 20°C thaw	% Cook loss, 5°C thaw	% Cook loss, 20°C thaw
Model	23	12.89**	0.22**	0.22**	38.45**	42.71**
Freezing rate (3)		95.40**	1.33**	0.40**	15.34**	48.40**
Formulation (5)		0.50	0.08*	0.41**	138.96**	143.25**
FR x Formulation (15)		0.52	0.04*	0.11**	9.57**	8.05**
Residual	96	0.34	0.02	0.03	2.63	2.90

*p<0.05; **p<0.01

Table 22. Mean squares of the analysis of variance for the water holding capacity of the nonfrozen, non-cooked patties

Source	d.f.	Water holding capacity (total area/meat area)
Model	5	50.95**
Freezing rate (0)		--
Formulation (5)		50.95**
FR x Formulation (15)		--
Residual	12	3.66

**p<0.01.

Table 23. Mean squares of the analysis of variance for percentage drip loss

Source	d.f.	% Drip loss
Model	23	23.05**
Freezing rate (3)		35.48**
Formulation (5)		63.29**
FR x Formulation (15)		7.15**
Residual	48	1.59

**p<0.01.

Table 24. Mean squares of the analysis of variance for percentage cooking loss from patties cooked non-frozen and frozen

Source	d.f.	% Cooking loss
Model	29	76.60**
Freezing rate (4)		157.71**
Formulation (5)		270.52**
FR x Formulation (20)		11.89**
Residual	120	3.23

**p<0.01.

Table 25. Mean squares of the analysis of variance for Warner-Bratzler shear values

Source	d.f.	W/B shear values (kgs)
Model	29	11.58**
Freezing rate (4)		10.60**
Formulation (5)		50.48**
FR x Formulation (20)		2.05**
Residual	420	0.50

**p<0.01.

Table 26. Mean squares of the analysis of variance for DNA concentration and total DNA content of the pattie drip fluid

Source	d.f.	DNA (mg/ml)	Total DNA (mg)
Model	23	3079.43**	242029.61**
Freezing rate (3)		4589.18**	137643.08*
Formulation (5)		6027.95**	685318.42**
FR x Form. (15)		1794.64**	115143.96**
Residual	48	141.66	46195.89

*p 0.05; **p 0.01.

Table 27. Mean squares of the analysis of variance for protein concentration of the pattie drip fluid

Source	d.f.	Protein (mg/ml)
Model	23	1679.76**
Freezing rate (3)		2712.53**
Formulation (5)		3093.41**
FR x Form. (15)		1101.99**
Residual	24	342.53

**p 0.01.

Table 28. Mean squares of the analysis of variance for the sensory evaluation of flavor, texture, juiciness and overall acceptability

Source	d.f.	Flavor	Texture	Juiciness	Overall acceptability
Model	29	4.20**	6.03**	7.87**	3.53**
Freezing rate (4)		4.40*	17.15**	21.32**	6.91**
Formulation (5)		7.55**	9.05**	13.17**	5.64*
FR x Formulation (20)		3.32*	3.05	3.86*	2.32
Residual	387	1.80	2.08	2.06	1.89

*$p<0.05$; **$p<0.01$.

Table 29. Means squares of the analysis of variance for color reflectance values

Source	d.f.	Color reflectance value
Model	29	112.18**
Freezing rate (4)		624.96**
Formulation (5)		127.05**
FR x Formulation (20)		5.91**
Residual	60	1.25

**p<0.01.

APPENDIX B

Ultraviolet Absorption Measurements of DNA

References: Fleck, A. and H. N. Monro. 1962. Biochim. Biophys. Acta 55: 55:571-583. Revised by Trenkle et al. (1978).

DNA determination

1. To a culture tube add .20 ml drip, .80 ml .4N KCl and 1 ml .2N KOH; mix.
2. Add 1 ml .08% indole and mix. To tissue blanks add 1 ml deionized water instead of indole.
3. Add 1 ml 4N HCl (ice cold); mix.
4. Heat in boiling water bath for exactly 15 minutes. Cool rapidly in ice bath.
5. Extract 3 times with 3.75 ml chloroform per extraction. Centrifuge for 10 minutes at 500 x g each time.
6. Measure O.D. at 490 mμ.

DNA standard: 500 μg/ml

Dissolve 25 mg of calf thymus DNA in .001N NaOH. Dilute to 50 ml volume with .001N NaOH.

Working standard

DNA (μg/ml)	dilution
10	1 ml in 50 ml
20	1 ml in 25 ml
30	3 ml in 50 ml
40	2 ml in 25 ml
60	3 ml in 25 ml

Dilute to volume with .4N KCl.

Biuret Protein Analysis

Reference: AOAC 1965. Official Methods of Analysis 10th ed. Association of Official Analytical Chemists, Washington, D.C.

Protein Determination

1. Add 1.0 ml of drip fluid to a 50 ml volumetric flask and make to volume with distilled water.
2. Pipette 1.0 ml of diluted sample solution to a clean glass test tube.
3. Add 4.0 ml of Biuret reagent; mix.
4. Allow color development for 30 minutes and read at 540 mμ.

Bovine Serium Albumin (BSA) standard: 10 mg/ml

Working standard

Concentration BSA, mg/ml	dilute
2	.2 ml BSA + .8 ml water
4	.4 ml BSA + .6 ml water
6	.6 ml BSA + .4 ml water
8	.8 ml BSA + .2 ml water

Lipid Staining

Reference: Humanson, G. L. 1972. Animal Tissue Techniques. W. H. Freeman Co., San Francisco.

Procedure

1. Mount frozen sections on slides: dry.
2. Rinse in 60% isopropanol: 30 seconds.
3. Stain in oil red 0: 10 minutes.
4. Rinse in 60% isopropanol: few seconds.
5. Wash in running water: 2-3 minutes.
6. Stain in Mayer hematoxylin: 2-3 minutes.
7. Wash in tap water: 3 minutes.
8. Blue in Scott solution: 3 minutes.
9. Wash in tap water: 5 minutes. Mount in glycerol jelly.

Results

lipids - orange-red or brilliant red

nuclei - dark blue